European Patent Office

SUPPLEMENTARY PARTIAL EUROPEAN SEARCH REPORT

under Rule 46, paragraph 1 of the European Patent Convention

Application Number EP 99 92 4258

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO 97 28821 A (APHTON CORP) 14 August 1997 (1997-08-14) * page 3, line 33 - page 4, line 2 * * page 4, line 9 - line 28 * * page 5, line 7 - line 33 * * page 7, line 16 - line 24 * * claims 1,2 * * figure 1 *	1-8	A61K39/385 C07K7/06 C07K7/08
X	* the whole document * --- -/--	9-29	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
A61K

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

The present partial European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

Place of search	Date of completion of the search	Examiner
MUNICH	17 April 2002	Irion, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03 82 (P04C23)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 99 92 4258

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	WATSON SUSAN A ET AL: "Pre-clinical evaluation of the gastrimmune immunogen alone and in combination with 5-fluorouracil/leucovorin in a rat colorectal cancer model." INTERNATIONAL JOURNAL OF CANCER, vol. 75, no. 6, 16 March 1998 (1998-03-16), pages 873-877, XP002194963 ISSN: 0020-7136 * page 873, right-hand column, paragraph 2 * * page 873, right-hand column, paragraph 4 * * page 874, left-hand column, paragraph 1 * * page 874, left-hand column, paragraph 8 - right-hand column, paragraph 1 * * page 877, right-hand column, paragraph 2 - paragraph 3 *	1-8	
X	* the whole document *	9-29	
Y	SUMII MASAHARU ET AL: "Regulation of antral peptides by administration of omeprazole to healthy men." AMERICAN JOURNAL OF GASTROENTEROLOGY, vol. 89, no. 11, 1994, pages 2033-2037, XP001068668 ISSN: 0002-9270 * abstract * * page 2033, left-hand column, paragraph 2 * * page 2034, right-hand column, paragraph 7 * * page 2035, right-hand column, paragraph 2 * * page 2036, left-hand column *	11, 13-25, 27-29	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

-/--

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 99 92 4258

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y	MCCLOY R F ET AL: "Pathophysiological effects of long-term acid suppression in man." DIGESTIVE DISEASES AND SCIENCES, vol. 40, no. 2 SUPPL., 1995, pages 96S-120S, XP001068385 ISSN: 0163-2116 * abstract * * page 104S, left-hand column, paragraph 4 * * page 103S, left-hand column, paragraph 4 - right-hand column, paragraph 1 * * page 102S, left-hand column, paragraph 1 * * page 100S, left-hand column, paragraph 5 - right-hand column, paragraph 1 *	11-29	
Y	FRESTON JAMES W: "Long-term acid control and proton pump inhibitors: Interactions and safety issues in perspective." AMERICAN JOURNAL OF GASTROENTEROLOGY, vol. 92, no. 4 SUPPL., 1997, pages 51S-57S, XP001068667 ISSN: 0002-9270 * abstract * * page 52S, right-hand column, paragraphs 2,4,5 * * page 51S, right-hand column, paragraphs 1,3,4 * * page 51S, left-hand column, paragraph 2 *	11-29	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C10)

P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
☎ +31 70 340 2040
TX 31651 epo nl
FAX +31 70 340 3016

Eur päisches Patentamt
Zweigstelle in Den Haag Recherchen-abteilung

Eu lan Pa Office
Branch at The Hague Search division

Office uropéen des brev ts
Département à La Haye Division de la recherche

Hale, Stephen Geoffrey
JY & GW Johnson,
Kingsbourne House,
229-231 High Holborn
London WC1V 7DP
GRANDE BRETAGNE

DUE: 4/1/03
3/31/03

RECEIVED

Datum/Date
31.01.03

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°.
ACP1EPO	99924252.2-2107-US9910734
Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire: APHTON CORP.	

COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

☐ Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

EUROPÄISCHES PATENTAMT · EUROPEAN PATENT OFFICE · OFFICE EUROPÉEN DES BREVETS · DEN HAAG · THE HAGUE · LA HAYE

REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

European Patent Office

SUPPLEMENTARY EUROPEAN SEARCH REPORT

Application Number
EP 99 92 4252

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	HALTER F ET AL: "EVALUATION OF A MONOCLONAL ANTI-GASTRIN ANTIBODY AS A TOOL FOR IMMUNONEUTRALIZATION OF GASTRIN DURING OMEPRAZOLE TREATMENT IN THE RAT" GASTROENTEROLOGY, vol. 96, no. 5 PART 2, 1989, page A194 XP009004160 ISSN: 0016-5085 * abstract * -----	1-14	A61K39/00 A61K39/385 A61K38/00 A61K31/44 A61K31/415 A61K31/425

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61K
A61P

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

4

Place of search	Date of completion of the search	Examiner
MUNICH	22 January 2003	Pilling, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C04)

(EW)

EVALUATION OF A MONOCLONAL ANTI-GASTRIN ANTIBODY AS A TOOL FOR IMMUNONEUTRALIZATION OF GASTRIN DURING OMEPRAZOLE TREATMENT IN THE RAT

F. Halter, F.Eigenmann, B.Flogerzi, L.Varga, J.Altorfer, H.Wong, J.H. Walsh, Gastrointestinal Unit, University Hospital, Inselspital Bern, Switzerland and CURE/VA Wadsworth and Dept. of Medicine, UCLA, Ca 90073

In the rat 5 day treatment with omeprazole at doses which induce complete anacidity is followed by a 5-10 fold increase in plasma gastrin (Inauen W. et al. GE; 1986: 90, 1471 and GE 1988, 95: 636). In the present study we assessed the potential of a monoclonal anti-gastrin antibody to neutralize drug induced hypergastrinemia. In preliminary experiments the acid response to intravenous infusion of 500 $ng.kg^{-1}$ of human gastrin-17 was completely abolished in the lumen perfused anesthetized rat following i.v. infusion of 10 $mg.kg^{-1}$ of the purified anti-gastrin-17 monoclonal antibody (Cure.GAS.93), but not following the control anti-gly-gastrin antibody (Cure.GL7.109-21) which has been shown not to react with gastrin-17 in the RIA. When the same dose of GAS.93 was intravenously applied on day 1 and 3 during the 5 day omeprazole study (40 $\mu mol.kg^{-1}$ body weight s.c. at 10 hrs each day) to 7 chronic gastric fistula rats high anti-gastrin binding capacities were maintained in the plasma throughout the study in GAS.93 treated animals (81.9±3.9 % (mean ±SEM) on day 1, 64.7±2.6 % on day 3 and 74.5±2.5 % on day 5). In 7 animals treated with GL7.109-21 the respective values were below 4 %. Plasma gastrin was measured by RIA before omeprazole and antibody injection and 3 and 5 days after the onset of the omeprazole/antibody treatment after heat inactivation of the antibody. In animals treated with GL7.109-21 the plasma gastrin levels rose from 30.8±5.8 $pmol.l^{-1}$ (mean±SEM) to 506.4± 66.3 (day3) and 483.0±53.7 (day5), and in GAS.93 treated animals from 24.4± 2.8 to 3387.1±397.3 and 5882.5±234.4 respectively.

Conclusion: The results demonstrate, that the Cure.GAS.93 antibody is capable to bind considerable amounts of plasma gastrin. Thus, this monoclonal anti-gastrin antibody may be a useful tool for prolonged immunoneutralization of endogenous gastrin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/385; C07 K 7/06, 08

US CL : 424/184.1, 194.1, 198.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 194.1, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN on line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THORNDYKE M., Identification and localozation of material with gastrin-like immunoreactivity in the neutral ganglion of a photochordate, Ciona intestinalis. Regulatory Peptides, Vol. 16, pages 269-279, 1986, see abstract	1-14
X --- Y	WATSON et al. Gastrimmune Raises Antibodies that Neutralize Amidated and Glycine extended Gastrin-17 and inhibit the growth of colon cancer. Cancer Research, 1996, Vol.56, pages 880-885. See abstract, Fig.2, p. 883, right column.	1,3-5,7,10-12,14 ----- 2,9,6,13
X --- Y	WATSON et al. Anti-gastrin antibodies raised by gastriimmune inhibit growth of the human colorectal tumor AP5. Int. J. Cancer, 1995, Vol. 61, pages 233-240; see abstract	1;3-5; 7, 10-12, 14 ----- 2,9,6,13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 AUGUST 1999

Date of mailing of the international search report

19 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

MICHAEL BORIN

for

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,023,077 A (GEVAS et al) 11 June 1991, see abstract, claims 1-8.	1-14
X	US 5,468,494 A (GEVAS et al) 21 November 1995, see abstract, claims 1-6.	1-14
X	US 5,609,870 A (GEVAS et al) 11 March 1997, see abstract, claims 1-5.	1-14
X	US 5,607,676 A (GEVAS et al) 04 March 1997, see abstract, claims 1-13.	1-14
X	US 5,622,702 A (GEVAS et al) 22 April 1997, see abstract, claims 1-4.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional search fees must be paid.

Group I, claims 1-4, 8-11, and claims 7, 14 in part, drawn to methods of use of G17 or G34 peptide coupled to immunogenic carrier.

Group II, claims 5,6, 12, 13, and claims 7, 14 in part, drawn methods of use of anti-G17 antibodies.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I, II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they do not have a the same or corresponding special technical features. The method of Group I employs G17 or G34 peptide conjugated to immunogenic carrier via a peptide link, whereas the method of Group II utilizes anti-G17 antibody. Therefore, the lack of unity is present because the linking technical feature is not a "special technical feature" as defined by PCT Rule 13.2.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: ACG2PCT

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US99/10751	14 May 1999 (14.05.99)	15 May 1998 (15.05.98)

Title of invention: Prevention and Treatment of Hypergastrinemia

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Aphton Corporation
Brickell Bay View Center
80 SW Eighth Street, Suite 2160
Miami, Florida 33130
United States of America

Telephone No.: 305-374-7338
Facsimile No.: 305-374-7615
Teleprinter No.:

State *(that is, country)* of nationality: United States of America
State *(that is, country)* of residence: United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Gevas, Philip C.
881 Ocean Drive, #23D
Key Biscayne, Florida 33149
United States of America

State *(that is, country)* of nationality: United States of America
State *(that is, country)* of residence: United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Grimes, Stephen
551 Rutgers Drive
Davis, California 95616
United States of America

State *(that is, country)* of nationality: United States of America
State *(that is, country)* of residence: United States of America

[x] Further applicants are indicated on a continuation sheet.

International application No.
PCT/US99/10751

Continuation of Box No. II **APPLICANT(S)**

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Karr, Stephen
2265 Halsey Circle
Davis, California 95616
United States of America

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Michaeli, Dov
21 Marina Vista Avenue
Larkspur, California 94939
United States of America

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
United States of America	United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Watson, Susan
5 Seatolla Close
Edwalton,
Nottingham NG2 6RB
Great Britain

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
United Kingdom	United Kingdom

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US99/10751

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Bak, Mary E.
Howson and Howson
Spring House Corporate Center
P.O. Box 457
Spring House, Pennsylvania 19477
United States of America

Telephone No.: 215-540-9200

Facsimile No.: 215-540-5818

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[x] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[ ] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/US99/10751

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

By: Mary E. Bak
Mary E. Bak
Attorney for Applicants

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MARY E. BAK
HOWSON & HOWSON
SPRING HOUSE CORPORATE CENTER
P.O.BOX 457
SPRING HOUSE, PA 19477

RECEIVED
JUN 12 2000

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	07 JUN 2000
Applicant's or agent's file reference: ACG2PCT	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/10751	14 MAY 1999	15 MAY 1998

International Patent Classification (IPC) or both national classification and IPC
IPC(7): A61K 39/385; CO7 K 7/06, 08 and US Cl.: 424/184.1, 194.1, 198.1

Applicant
APHTON CORPORATION

ENTERED
DUE 8-7-00

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 28 SEPTEMBER 2000.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MICHAEL BORIN James R. Matthews
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

WRITTEN OPINION

International application No.
PCT/US99/10751

I. Basis of the opinion

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages (See Attached) , as originally filed
pages , filed with the demand
pages , filed with the letter of

[X] the claims:
pages (See Attached) , as originally filed
pages , as amended (together with any statement) under Article 19
pages , filed with the demand
pages , filed with the letter of

[X] the drawings:
pages (See Attached) , as originally filed
pages , filed with the demand
pages , filed with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:

[X] the description, pages none

[X] the claims, Nos. none

[X] the drawings, sheets/~~fig~~ none

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

WRITTEN OPINION

International application No.
PCT/US99/10751

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	none	YES
	Claims	1-14	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-14	NO
Industrial Applicability (IA)	Claims	1-14	YES
	Claims	NONE	NO

2. citations and explanations

The subject matter of claims 1,3-5,7,10-12,14 does not appear to have an inventive step on account of the disclosure of documents 2, 3 (Watson et al.), cited in the ISR

Watson, 95 and 96 references teach immunogen, Gastrimune, which is composed of a portion of G17 linked to immunogenic carrier, such as diphteria toxoid. This composition was used in rising anti-G17 antibodies; the later reduced gastrin level in vivo. Immunization with anti-G17 antibodies inhibits a rat colon tumor.

In regard to claims 3,10 the immunogenic carriers claimed therein are known in the art. see documents 4-8 described below.

In regard to claims 4,13, it would be obvious to apply anti-hypergastrinemia to any disorder condition requiring decrease in gastrin level.

The subject matter of claims 1-14 does not appear to have an inventive step on account of the disclosure of documents 4-8 (patents of Gevas et al.), cited in the ISR

Gevas et al ('077,'494,702,'676,'870 patents) teach immunogens comprising immunogenic carrier (such as diphteria toxoid) conjugated to a fragment of G17. The conjugation is via a spacer which, in particular, can contain Ser residue (see '494, claim 7). The conjugate is used for treatment of gastrin-induced disorders, e.g., ulcers and tumors. The conjugate is also used for production of anti-gastrin antibodies.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

I. BASIS OF OPINION:

This opinion has been drawn on the basis of the description,
pages, 1-22, as originally filed.
pages, NONE, filed with the demand.
and additional amendments:
NONE

This opinion has been drawn on the basis of the claims,
numbers, pages 23-24, as originally filed.
numbers, NONE, as amended under Article 19.
numbers, NONE, filed with the demand.
and additional amendments:
NONE

This opinion has been drawn on the basis of the drawings,
sheets, 1-7, as originally filed.
sheets, NONE, filed with the demand.
and additional amendments:
NONE

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MARY E. BAK
HOWSON & HOWSON
SPRING HOUSE CORPORATE CENTER
P.O.BOX 457
SPRING HOUSE, PA 19477

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	05 OCT 2000

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
ACG2PCT	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/10751	14 MAY 1999	15 MAY 1998

Applicant
APHTON CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MICHAEL BORIN
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ACG2PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/10751	International filing date *(day/month/year)* 14 MAY 1999	Priority date *(day/month/year)* 15 MAY 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 39/385; CO7 K 7/06, 08 and US Cl.: 424/184.1, 194.1, 198.1		
Applicant APHTON CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14 DECEMBER 1999	07 SEPTEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL BORIN Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/10751

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages none

[X] the claims, Nos. none

[X] the drawings, sheets/~~fig~~ none

5. [X] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/10751

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	none	YES
	Claims	1-14	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-14	NO
Industrial Applicability (IA)	Claims	1-14	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

The subject matter of claims 1,3-5,7,10-12,14 does not appear to have an inventive step on account of the disclosure of documents 2, 3 (Watson et al.), cited in the ISR

Watson, 95 and 96 references teach immunogen, Gastrimune, which is composed of a portion of G17 linked to immunogenic carrier, such as diphteria toxoid. This composition was used in rising anti-G17 antibodies; the later reduced gastrin level in vivo. Immunization with anti-G17 antibodies inhibits a rat colon tumor.

In regard to claims 3,10 the immunogenic carriers claimed therein are known in the art. see documents 4-8 described below.

In regard to claims 4,13, it would be obvious to apply anti-hypergastrinemia to any disorder condition requiring decrease in gastrin level.

The subject matter of claims 1-14 does not appear to have an inventive step on account of the disclosure of documents 4-8 (patents of Gevas et al.), cited in the ISR

Gevas et al ('077,'494,702,'676,'870 patents) teach immunogens comprising immunogenic carrier (such as diphteria toxoid) conjugated to a fragment of G17. The conjugation is via a spacer which, in particular, can contain Ser residue (see '494, claim 7). The conjugate is used for treatment of gastrin-induced disorders, e.g., ulcers and tumors. The conjugate is also used for production of anti-gastrin antibodies.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-22, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the claims,
page(s) pages 23-24, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the drawings,
page(s) 1-7, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

5. (Some) amendments are considered to go beyond the disclosure as filed:
NONE

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZW Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☒ AE United Arab Emirates .....
☒ CY Cyprus .....
☒ ZA Republic of South Africa .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

"Continuation of Box No. IV"

Kita, Stanley B.
Smith, George A., Jr.
Kodroff, Cathy A.
Bak, William

All above attorneys are members of the firm of Howson and Howson.
Address of all is indicated in Box IV.

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 15 May 1998	60/085,714	United States of America		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /USPTO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	5
description (excluding sequence listing part)	22
claims	2
abstract	1
drawings	12
sequence listing part of description	2
Total number of sheets	44

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: transmittal letter to USRO

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

By: Mary E. Bak
Mary E. Bak
Attorney for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

214

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 February 2000 (14.02.00)	
International application No. PCT/US99/10751	**Applicant's or agent's file reference** ACG2PCT
International filing date (day/month/year) 14 May 1999 (14.05.99)	**Priority date** (day/month/year) 15 May 1998 (15.05.98)
Applicant GEVAS, Philip, C. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 December 1999 (14.12.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia RANAIVOJAONA
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 39/385, C07K 7/06, 7/08

A1

(11) International Publication Number: WO 99/59631

(43) International Publication Date: 25 November 1999 (25.11.99)

(21) International Application Number: PCT/US99/10751

(22) International Filing Date: 14 May 1999 (14.05.99)

(30) Priority Data:
60/085,714 15 May 1998 (15.05.98) US

(71) Applicant *(for all designated States except US)*: APHTON CORPORATION [US/US]; Brickell Bay View Center, Suite 2160, 80 S.W. Eighth Street, Miami, FL 33130 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GEVAS, Philip, C. [US/US]; 881 Ocean Drive #23D, Key Biscayne, FL 33149 (US). GRIMES, Stephen [US/US]; 551 Rutgers Drive, Davis, CA 95616 (US). KARR, Stephen [US/US]; 2265 Halsey Circle, Davis, CA 95616 (US). MICHAELI, Dov [US/US]; 21 Marina Vista Avenue, Larkspur, CA 94939 (US). WATSON, Susan [GB/GB]; 5 Seatolla Close, Edwalton, Nottingham NG2 6RB (GB).

(74) Agents: BAK, Mary, E. et al.; Howson and Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).

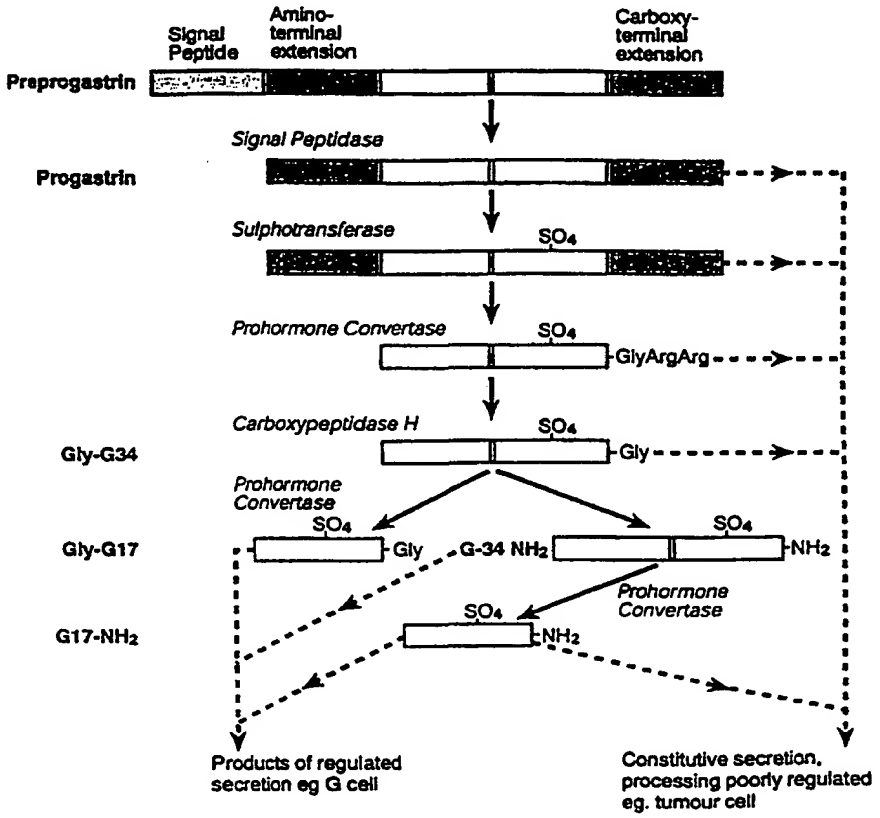
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PREVENTION AND TREATMENT OF HYPERGASTRINEMIA

(57) Abstract

Serum–associated hypergastrinemia is treated by administration of gastrin active or passive immunization. An anti–gastrin immunogenic composition comprising a gastrin G17 or G34 peptide fragment which is amino acid spacer–linked to an immunogenic carrier, is administered so as to effectively neutralize the circulating gastrin hormone, and moreover, inhibit autocrine activity by progastrin such as Gly–extended G17, and amidated G17, in patients with pernicious anemia. Moreover, the method includes administration of a therapeutically effective amount of anti–G17 or anti–G34 antibodies which may be in humanized form. Finally, the method provides ameliorating treatment of hypergastrinemic effects of proton pump inhibitors or H_2 histamine receptor blocking agents or antagonists, in addition to treatment of hypergastrinemia caused by diseases such as pernicious anemia.



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PREVENTION AND TREATMENT OF HYPERGASTRINEMIA

FIELD OF THE INVENTION

The invention relates to the prevention and/or treatment of hypergastrinemia by immunological control of gastrin levels.

BACKGROUND OF THE INVENTION

In humans, treatment with proton pump inhibitors, infection with *Helicobacter pylori* and pernicious anemia account for the majority of cases of hypergastrinemia. Marked hypergastrinemia is seen in the relatively infrequent Zollinger-Ellison Syndrome (ZES). One of the direct effects of hypergastrinemia is, of course, high secretion rates of gastric acid in the stomach.

Around 90% of patients with pernicious anemia (PA) are hypergastrinemic and total gastrin levels can be up to forty times higher than normal levels. A recent study by Varro et al, J. Clin. Invest., 95:1642-1649 (1995) has demonstrated that the hypergastrinemia associated with PA is composed of substantially elevated amidated gastrin, and moderate elevations in the precursors progastrin and glycine extended G17 (gly-G17).

Gastrin peptides are the products of extensive post-translational processing as outlined in Fig. 1. The first translation product of a single mRNA of 0.7 kb is the 101 amino acid precursor preprogastrin. This peptide is translocated into the lumen of the rough endoplasmic reticulum where it is converted into the progastrin peptide. The progastrin moves through the secretory pathway to the golgi stack, and is sulfated at Tyr^{87} prior to endoproteolytic cleavage and maturation in the secretory granules. As a consequence, progastrin is processed to give G34 from dibasic cleavage at sites Arg^{57} Arg^{58} and Arg^{94} Arg^{95} and to give G17 from dibasic cleavage at sites Lys^{74} Lys^{75} and Arg^{94} Arg^{95}. While prehormone convertase 2 (PC2) producing G17 is located primarily in the gastric antrum, the prohormone convertases PC1/PC3 producing G34 are located in the duodenum. The dibasic cleavage residues are removed by carboxypeptidase H (CPH) producing Gly^{93} extended gastrins serving as substrates for the amidation enzyme, PAM (peptidylglycine α-amidating monoxgenase).

Amidated G17 gastrin appears to be a conversion product of G34 NH_4 which is an amidation product of Gly-G34. Gly G17 has been thought as a second endpoint of progastrin processing.

Gastrin effects on tumor cells are via endocrine, paracrine, autocrine and intracrine pathways (Fig. 2) where, however, not all receptor types have been characterized. It is known that exogenous gastrin stimulates gastric and colorectal tumor cells and tumor cell lines.

Most PA patients have endocrine hyperplasia in the gastric corpus and fundus. There is a significant positive correlation between the degree of hypergastrinemia and the number of enterochromaffin-like (ECL) cells. However, the histological type of ECL cell hyperplasia is not dependent on the degree of hypergastrinemia as there is no significant difference in the gastrin levels in patients with linear or nodular hyperplasia. Once diagnosed, despite continuing elevated gastrin levels, the ECL cell hyperplasia appears to remain stable.

The prevalence rate of gastric carcinoid in endoscopically examined PA patients' ranges from 4 to 7%. Patients with carcinoid are diagnosed as having PA 10 years earlier than the average PA patient. This precedes the diagnosis of the carcinoid tumor by a mean of 10 to 12 years. There is no predictive sign for the occurrence of gastric carcinoids in patients with PA, though mean serum gastrin levels are higher in carcinoid compared to ECL hyperplasia [Brinton et al, Brit. J. Cancer, 59:810-813 (1989)]. The stimulus to undergo malignant transformation is thought to be provided by the autoantibodies present. The tumors appear hormonally dependent. Patients who have undergone antrectomy in order to correct hypergastrinemia, have demonstrated disappearance of hyperplastic polyps, carcinoids or agyrophil micronodules diagnosed endoscopically and/or histologically. The demonstration of complete resolution of ECL-cell carcinoids after antrectomy in some patients confirms the potency of hypergastrinemia as a trophic principle for fundic ECL-cells.

In PA, evidence for an effect of the associated hypergastrinemia on other cancers in the gastrointestinal tract comes only from epidemiological studies. Several studies have looked at the incidence of colorectal cancer in PA. A slight increase in the prevalence of colorectal cancer in the first five years after diagnosis of PA has been reported [Talley et al, Annals Int. Medicine, 111:738-742 (1989)].

Studies have also demonstrated an increased prevalence (approximately 7%) of gastric adenocarcinoma in PA. Hypergastrinemia may be responsible for this observed increase. Even though a correlation to serum gastrin levels cannot be found in the majority of patients with gastric cancer, a correlation to chronic atrophic gastritis is always present.

The epidemiological studies have failed to show a consistent increase in the incidence of colorectal cancer in PA. This may be due to the deficient design of the studies. In each analysis the PA patients were compared to unscreened controls from the general population. A proportion of the controls may be expected to be hypergastrinemic due to either *Helicobacter pylori* infection, atrophic gastritis or following administration of a proton pump inhibitor. The apparent failure to show an increase in the incidence of tumors could be explained by the action of gastrin – it acts as a mitogen not a mutagen. However, as gastrin promotes the proliferation of the normal colonic mucosa, there may be an increased chance of a spontaneous mutation, which would affect tumor incidence.

A single study performed recently has looked at the proliferation rate of cells of the normal colon in patients with PA compared to normal controls [Talley et al, cited above]. The control patients were normogastrinemic and had no colonic abnormalities assessed by colonoscopy or barium enema. Using 5'-bromodeoxyuridine to provide a proliferation index, the percentage of proliferating cells in the entire crypts was similar in both groups. In the PA group there was a significantly higher labeling frequency in the upper two fifths of the glands ($p<0.01$). Movement of the proliferative compartment is seen in individuals at high risk of cancer.

Long-term treatment with omeprazole is known to induce ECL cell hyperplasia which is related to the serum gastrin level. Chronic hypergastrinemia-related carcinoid tumors of the stomach have been reported in certain animals test subjects, e.g. rats, although not yet confirmed in the human [Sobhani et al, Gastroenterology, 105:22-30 (1993)].

Proton pump inhibitors cause a twofold to fourfold increase in fasting and postprandial plasma gastrin concentrations. The increase in fasting hypergastrinemia occurs within a few months of starting therapy. Occasionally, markedly elevated gastrin levels (10 fold) may develop during long term treatment with omeprazole, e.g.,

20-60mg/day. Gastrin levels stabilize after a few months of therapy even if the dose of omeprazole is decreased from 40mg to 20 mg daily [Sontag et al, Gastroenterology, 102:109-118 (1992)].

The growth of gastric endocrine cells has been extensively monitored in patients treated with 20 to 40mg omeprazole daily for up to eight years. No significant quantitative changes of the antral G- and D- cells have been found even after years of high-dose omeprazole treatment. In comparison, the G-cell volume in rats doubled both qualitatively and quantitatively after four weeks of treatment with omeprazole [Tielemans et al, Gastroenterology, 96:723-729 (1989)]. Only patients with the highest serum gastrin levels (>240 pg/ml, four times the upper limit of normal) showed an increase in gastric ECL-cell volume density between the third and fifth year of therapy. This data supports earlier findings that an increase of the ECL-cell volume density is correlated to elevated fasting serum gastrin levels. In addition, linear and nodular hyperplasia was confined to the group of patients with the highest serum gastrin levels. Dysplasia was not been seen in any patient.

A correlation between different grades of atrophy of the oxyntic mucosa and ECL cell growth has been established. In patients receiving 40mg of omeprazole daily for eight years, it was found that the prevalence of micronodular hyperplasia in superficial corpus gastritis was low, e.g., 3.6%, increasing to 19.6% in interstitial gastritis and to 48% in atrophic gastritis. This relationship between atrophic gastritis and micronodular hyperplasia may partially be explained by condensation of the endocrine cells caused by atrophy of the gastric glands and thus may not represent true hyperplasia. Therefore, excessive long-lasting hypergastrinemia induced by omeprazole leads to only linear and simple hyperplasia. In patients with atrophic gastritis or those with a genetic predisposition, hypergastrinemia gives rise to micronodular hyperplasia under the chronic treatment.

ECL hyperplasia in animal models occurs following the administration of omeprazole. The relative growth of both exocrine and endocrine cells produced by hypergastrinemia varies between species. For example, administration of omeprazole, (400μmol/kg, 14mg/kg) to mice for 10 weeks resulted in a threefold increase in plasma gastrin during treatment. Furthermore, the stomach weight increased by 34% and the ECL density by 37% at the end of treatment. The same dose has been found in rats to

increase the gastrin levels 10-fold, resulting in the same general trophic effect (increase of stomach and mucosal weight) as in mice, but the ECL cell density increases by about 300%. The significance of this imbalance in the trophic effect of gastrin on the exocrine cells and ECL cells for the development of carcinoids in rats is not known.

Several studies have investigated the effect of hypergastrinemia on normal colonic epithelial cells. The majority of these studies have induced hypergastrinemia by omeprazole administration or as a result of antral exclusion and have produced conflicting results. The effect of long-term (1-year) treatment of female rats with high dose daily omeprazole (400μmol/kg, 14mg/kg) which led to 15 fold increase in gastrin compared to controls was examined [Sundler et al, in Proc. The First Interntional Symposium on Omeprazole, K. O. Borg et al eds, AB Hässle (1986)]. The mucosal thickness of the colonic mucosa and the number of chromogranin-A-containing endocrine cells were unaffected by the omeprazole-induced hypergastrinemia. However, the same animals developed a modest and stable antral gastrin cell hyperplasia. Similarly, Oscarson demonstrated that long-term changes in endogenous gastrin concentration produced by fundectomy (resulting in a 3.5 fold gastrin elevation) did not result in colonic mucosal trophic effects.

In contrast, significantly enhanced proliferation of colonic mucosa in omeprazole treated rats compared to controls was demonstrated [Pawlikowski e tal, Hormone & Metab. Res., 21:89-91 (1989)]. Short term hypergastrinemia induced colonic mucosal proliferation as well as chronic endogenous hypergastrinemia were demonstrated in rats [McGregor et al, Annals Surg., 195:219-223 (1982)]. Chronic hypergastrinemia was achieved by antral exclusion and short term hypergastrinemia was achieved by pentagastrin administration (2mg/kg) every 12 hours for 48 hours prior to sacrifice. Tissue content and synthesis of DNA, RNA, and protein were all markedly increased by both endogenous gastrin and exogenous pentagastrin. The stimulation by gastrin was significantly stronger than that of pentagastrin. Using the metaphase-arrest technique it has also been have shown that an enhanced mitotic activity of the colonic mucosal cells in rats treated with omeprazole compared to controls [Lewinski et al].

More compelling evidence for a trophic role of gastrin has been provided by the development of gastrin deficient transgenic mice. These mice are incapable of

producing gastrin mRNA and the gastrin peptide. This deficiency has allowed studies on the effect of gastrin on the growth and development of the gastrointestinal tract.

Combining histology and immunohistochemical techniques, together with bromodeoxyuridine incorporation, the effect of exogenous gastrin on colonic architecture was assessed. The gastrin deficient mice had histologically normal colons. A decreased proliferation labeling index (2.97% ± 0.52%) was noted in such mice compared with wild-type animals (4.71% ± 0.44%; $P < 0.01$). The conclusion from these observations is that gastrin is trophic for the normal colonic mucosa.

According to Tang et al. (1996) carcinoid tumors from the Mastomys rodent during progression lose response to exogenous hypergastrinemia but have up-regulated expression of TGFÜ. As TGFÜ autocrine pathway potentially acts in a co-operative way with the gastrin autocrine pathway [Howell et al, (1997)], the lack of response of the carcinoids to exogenous gastrin may reflect the increasing activity of the gastrin autocrine pathway. The gastrin gene is apparently activated to rather a lower extent in adenomas than adenocarcinomas.

The conflicting results produced by the above studies may in part be explained by Wang et al, J. Clin. Invest., 98:1918-1929 (1996), in which evidence was provided that progastrin, once thought to be an inert precursor, also has a trophic effect on colonic mucosa. The study included the use of transgenic mice containing a human gastrin (hGAS) minigene, that expresses abundant human gastrin mRNA and human progastrin in the liver. The hepatocytes are unable to process this peptide to the mature amidated form, resulting in markedly elevated serum progastrin levels and normal amidated gastrin levels. The result was a marked increase in the bromodeoxyuridine labeling index of the colon, but not the gastric mucosa, in hGAS mice compared to age-matched, wild- type control mice. This study suggests that progastrin may contribute to colonic mucosal proliferation *in vivo*. Therefore, in conditions of hypergastrinemia not only may the degree of hypergastrinemia be important but the particular gastrin peptide which is elevated may also play a significant role. Normal colonic epithelial cells do not express classical gastrin/CCKB receptors so the action of gastrin must be mediated by an uncharacterized receptor that mediates the action of gastrin precursors.

As stated above, gastrin acts as a mitogen, and thus would not be expected to cause a cell to mutate. This hypothesis which has been confirmed in transgenic hGAS mouse studies. However, if the mucosa has an enhanced proliferation rate, there may be an increased chance of sporadic mutation. The only example of malignant change in animal models occurring in the presence of hypergastrinemia is carcinoid in rats following long term omeprazole administration. Although this finding is particular to rats, and no other animal model produces spontaneous carcinoids, it was felt that omeprazole may have a direct carcinogenic effect. However, the proton pump inhibitor class of drugs that produce hypergastrinemia, ECL hyperplasia and ECL carcinoids in the rat, has tested negatively for genotoxicity. Subsequent studies have shown that it is not a specific drug that leads to carcinoid formation; carcinoids can also be produced by feeding with 2000mg/kg ranitidine, loxitidine, the hypolipidemic agent clofibrate and by 75% corpectomy, all of which produce hypergastrinemia. The mediator role of gastrin was confirmed when it was shown that antrectomy in rats prevents omeprazole induced ECL cell hyperplasia. The formation of carcinoids in rats simply in the presence of hypergastrinemia may be due to their genetic background.

There is no reported evidence of hypergastrinemia producing spontaneous tumors at other sites in the gastrointestinal tract. In humans, it is evident that an additional factor may be required for ECL cells to progress from simple hyperplasia to carcinoid. In PA, the additional factor is possibly supplied by the presence of autoantibodies.

Once the cell has been transformed, exogenous gastrin can continue to promote growth. This effect may be enhanced by gastrin/CCKB receptors which are expressed de novo on adenomas. The exact point in the adenoma-carcinoma transformation sequence at which the gastrin/CCKB receptor and autocrine gastrin are expressed is not yet known. Hypergastrinemia may increase this transforming progression through the stages of the adenoma-carcinoma sequence.

In addition, treatment with agents directed against excess production of gastric acid has been found to induce parietal cell hyperplasia and hypertrophy. Recent cases were reported to suggest a correlation between gastric acid-inhibitory treatment by either proton pump inhibitors, such as omeprazole, lansoprazole, or histamine H_2 receptor inhibiting agents, such as ranitidine or cimetidine, and the occurrence of

fundic gland polyps (FGP).

A therapeutic method for selectively immunologically neutralizing the biological activity of the gastrin hormone would provide an effective means to control or prevent the physiopathological changes resulting from hypergastrinemia.

As disclosed in co-assigned U.S. Patents Nos. 5,609,870; 5,607,676; 5,622,702; 5,468,494; and 5,023,077, immunization against the G17 and G34 gastrin forms can effect neutralization of serum gastrin. The immunogenic constructs of this invention include an aminoterminal (1-9) G17 peptide or an aminoterminal (1-6) G34 peptide conjugated via a peptide spacer to an immunogenic carrier. The preferred G17 sequence is pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1] and the preferred G34 sequence is pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys [SEQ ID NO: 2]. The preferred spacer in both constructs is a Ser-peptide (Ser-Ser-Pro-Pro-Pro-Pro-Cys [SEQ ID NO: 3]). The preferred immunogenic carrier is diphtheria toxoid, tetanus toxoid, keylimpet hemocyanin, and bovine serum albumin (BSA). The gastrin immunogen is defined as a conjugate of the pGlu- Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1] peptide sequence, with an amino acid spacer linked to an immunogenic carrier. The preferred gastrin immunogen is defined as a conjugate of the (1-9) amino terminal (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1]) peptide which is linked by peptide spacer to diphtheria toxoid. It is further known that the gastrin immunogen preparation is also effective for inhibiting the incompletely processed or progastrin type gastrin precursors which may be bound to the cell membrane of a gastrin producing cell.

There is a need in the art for compositions and methods to effectively treat hypergastrinemia.

SUMMARY OF THE INVENTION

The present invention is directed to the treatment for control or prevention of gastrointestinal disorders such as hypergastrinemia by administering a gastrin immunogen preparation to an afflicted mammal or human.

A preferred embodiment of the treatment is directed to the control or prevention of hypergastrinemia due to pernicious anemia.

Another preferred embodiment of this invention is directed to the treatment for control or prevention of gastrointestinal side effects due to antiulcer agents such as proton pump inhibitors or histamine H_2 receptor blocking agents or antagonists.

It is another preferred embodiment of this invention to treat hypergastrinemia related to colorectal disorders or diseases by immunization with gastrin immunogen against gastrin peptide G17, G34, amidated gastrin and progastrin. In this context, the anti-G17 immunogen as described in U. S. Patent Nos. 5,609,870; 5,468,494; 5,785,970 and in the co-assigned patent application 08/798,423 has been found to provide an effective agent to stimulate anti-G17 antibodies which cross-react with Gly extended G17 (G17-Gly), amidated G17 (G17 NH_2) so as to be suitable for treating gastrointestinal tumors which are responsive to these gastrin peptides. The '423 application is incorporated herewith by reference in its entirety.

It is a special advantage of the present invention to provide a specific immunogen or antibody to target the specific protein which results in hypergastrinemia. For example, Gly G17 and G17 NH_2 can be neutralized with an anti-G17 immunogen composition, such as G17 (1-9) Ser DT, while G34 can be neutralized with anti-G34 (1-17) immunogens.

Moreover, G17 and G34 can be neutralized by anti-G34 (13-22) and anti-G34 (17-31) immunogens which generate antibodies able to cross-react with both gastrin epitopes. Passive immunization can be effected by the specific antibodies generated by immunogens against the various G17 and G34 epitope. These antibodies will either react specifically and separately with the G17 or G34 epitopes or react with both such gastrin epitopes together.

It is an especially preferred embodiment of this invention to treat or pre-treat with gastrin immunogen-type immunization a patient or mammal who is under chronic or long term treatment with the proton pump inhibitor, omeprazole or lansoprazole. A further embodiment provides passive immunization with anti-G17 antibodies which may be humanized to treat hypergastrinemia. A perfected combination treatment of hypergastrinemia and concomitant excess product of gastric acid involves administration of proton inhibitors or H_2 histamine receptor blockers.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the processing of the gastrin precursor to the mature gastrin forms;

Fig. 2 illustrates the various pathways of gastrin activity;

Fig. 3 illustrates the structural aspects of a gastrin immunogen.

Fig. 4 illustrates bound gastrin (median) in normal and hypergastrinemic subjects (the immunogen control group did not have bound serum gastrin above the 10 pg/ml detection limit).

Fig. 5 illustrates percentage survival of experimental hypergastrinemia mice treated with gastrin immunogen compared to controls.

Fig. 6 depicts the mean proliferation index of gastrin associated tumors in Min mice under treatment with gastrin immunogen.

Fig. 7 illustrates the timed levels of antibody in Min mice immunized with G17 (1-9):DT.

Fig. 8 compares the Min mouse anti-G17 (1-9):DT antibody levels in response to hG17-DT immunogen + vehicle, hG17-DT immunogen + omeprazole, vehicle only, omeprazole only, positive and negative controls.

Fig. 9 compares Min mouse serum G17 levels when immunized with 1) hG17-DT immunogen plus vehicle (Free G17) , 2) plus vehicle (Bound G17), 3) plus omeprazole (free G17) and 4) plus omeprazole (Bound G17).

Fig. 10 illustrates the percent animals surviving after treatment with oral vehicle plus blank immunogen (n=22); omeprazole (n=18) plus blank immunogen (n=30), oral vehicle plus gastrin immunogen (n=18); and omeprazole hG17-DT immunogen (n=30).

Fig. 11 shows the displacement of labelled G17 from anti-N-terminal gastrin (from rabbit anti-human G7 antiserum) by G17, Gly-G17, and G34 as described in Example 5.

Fig. 12 shows the displacement of labelled G17 from anti-C-terminal gastrin (from rabbit anti-human G7 antiserum) by G17, Gly-G17, and G34 as described in Example 5.

DETAILED DESCRIPTION OF THE INVENTION

As described above, immunogenic constructs useful in this invention include an aminoterminal (1-9) G17 peptide or an aminoterminal (1-6) G34 peptide conjugated via a peptide spacer to an immunogenic carrier. The preferred G17 sequence is pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1] and the preferred G34 sequence is pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys [SEQ ID NO: 2]. The preferred spacer in both constructs is a Ser-peptide (Ser-Ser-Pro-Pro-Pro-Pro-Cys [SEQ ID NO: 3]). The preferred immunogenic carrier is diphtheria toxoid, tetanus toxoid, keylimpet hemocyanin, and bovine serum albumin (BSA). The gastrin immunogen is defined as a conjugate of the pGlu- Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1] peptide sequence, with an amino acid spacer linked to an immunogenic carrier. The preferred gastrin immunogen is defined as a conjugate of the (1-9) amino terminal (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu [SEQ ID NO: 1]) peptide which is linked by peptide spacer to diphtheria toxoid.

In addition to the above-named anti-gastrin immunogens, one of the generating antibodies binding to both G17 and G34 comprises a conjugate of the 7 amino acids of C-terminal G17 amino acid sequence 11-17-DT. This sequence is E-A-Y-G-W-M-D-NH_2 [SEQ ID NO: 4]. An inhibition of G34 and G17 induced gastric acid production in perfused rat stomachs was observed after an intravenous injection of 1ml of rabbit Anti-C terminal G17 (11-17)-DT antisera.

In order to explore whether gastrin may promote progression to malignancy in existing pre-malignant conditions, studies were undertaken, for example, in the multiple intestinal neoplasia or so-called *Min* mouse model of familial adenomatous polyposis (FAP). The mice have a germline mutation in their APC gene which leads to multiple intestinal neoplasia. Hypergastrinemia which was induced by use of the proton pump inhibitor, omeprazole, has been now found to increase progression to malignancy in *Min* mice, reducing their median survival from approximately 10 weeks to 6 weeks. Examination of the proliferation of tumors from *Min* mice exposed to elevated gastrin levels revealed by bromodeoxyuridine incorporation that proliferation was increased.

In addition to the proliferative effects of serum-associated gastrin, acting in an endocrine manner to increase proliferation, expression of the gastrin gene has also been shown in the colonic mucosa in pre-malignant condition. In the transgenic APC1628 mouse, the gastrin gene is activated in both the normal colonic mucosa and the malignant epithelium [Smith et al, Brit. J.Surg., 84:706 (1997)]. This has recently been confirmed by applicants by both immunocytochemistry and at the gene level in the *Min* mouse. In addition, activation of the gastrin gene has been found in human adenomas [Smith et al, (1997) cited above]. Thus, a gastrin-mediated autocrine/paracrine pathway may also be operational in the pre-malignant scenario.

Effect of gastrin neutralization on the progression of the adenoma:carcinoma sequence in the *Min* mouse model of familial adenomatous polyposis has been observed. Specifically, both serum-associated gastrin and gastrin present with the colonic epithelium may contribute towards the progression cascade in the *Min* mouse model of FAP. Accordingly, the effect of gastrin neutralization on *Min* mouse survival was determined.

The *Min* mice used in the study were bred within the Academic Unit of Cancer Studies at Nottingham University (U.K.) on a C57/BL background. As the homozygous state is lethal and female *Min* mice do not lactate, a *Min* heterozygote is bred with a female wild type and 1:4 offspring have the *Min* genotype. The *Min* positive mice were then placed into each arm of the therapy on an ongoing basis.

The immunization with hG17-DT immunogen (G-17 conjugate) (Fig. 3), is effective in neutralizing circulatory gastrin levels as well as tissue bound precursors or incompletely processed progastrin.

Using exogenous anti-G17 antibodies which can be in humanized form, a patient can be preimmunized against hypergastrinemia or hypergastrinemic effects caused by treatment with proton inhibitors (omeprazole, lansoprazole, or pantoprazole) or H_2 receptor blockers (ranitidine, cimetidine, formatidine or nizatidine). Humanized antibodies may be prepared by techniques known in the art.

The hG17-DT conjugated immunogen or method of preparation are disclosed in U.S. Patent No. 5,609,870, U.S. Patent No. 5,468,494 and U.S. Patent No. 5,023,077 which are incorporated by reference in this specification in their entirety. See, also, Examples 3 and 4 below.

The endpoint of the treatment, which has previously been validated by Moser et al. (1990), is at the terminal stage of the disease, when the mice have a large tumor burden, blood is lost in the stools, and the animals become anemic.

The following examples are provided for illustration only.

Example 1

Gastrin neutralization was achieved by using an immunogen, i.e. the gastrin immunogen preparation, which is composed of the amino terminal domain of gastrin-17 linked, via an amino acid spacer, to diphtheria toxoid which acts as the immunogenic carrier. The antibodies raised by virtue of the design of the immunogen, cross-react with both amidated and glycine-extended gastrin-17, known proliferative forms of gastrin. *Min* mice were immunized s.c. with the hG17-DT immunogen (100 mg/mouse) at week 4, with subsequent injections at 3 weekly intervals. Serum antibody titres are known to rise within 2 weeks of the first immunization at levels with an antigen binding capacity of $> 10^{-9}$M. The hG17-DT immunogen was administered to mice at 4 weeks of age to examine its effect on mice with an established tumor burden. Control mice received immunogen constituents without the active peptide.

The presence of anti-gastrin antibodies within the serum of gastrin immunogen-immunized mice was confirmed by using an ELISA capture assay. To confirm the presence of antibody-bound gastrin, serum was taken from immunized mice, antibody:antigen complexes were purified, uncomplexed by boiling and the bound gastrin measured by RIA.

Bound gastrin levels were not measurable in animals immunized with control immunogen. The bound gastrin levels in the gastrin immunogen-immunized mice were 37pg/ml. In hypergastrinemic mice, with a 3-4 fold increase in serum gastrin levels, the bound gastrin levels was 148.3pg/ml which highlights the capacity of the antibodies raised in the serum for gastrin neutralization (Fig. 5).

There was a significant effect on survival, with the median survival in the control immunogen treated group being 8 weeks compared to 15 for the gastrin immunogen treated mice (Fig. 6). Log rank test p=0.0017. However, there is a sharp decline in the survival of the mice after 14-15 weeks.

One hour prior to termination, mice were injected with DNA analogue bromodeoxyuridine in order to determine the comparative in situ proliferation activity. The samples were formalin fixed, paraffin embedded, sections were cut and stained with an anti-bromodeoxyuridine antibody. There was no significant difference in the proliferation of crypts from both the small and large intestine due to gastrin immunogen treatment (Fig. 7). The proliferation of the small intestinal tumors were significantly inhibited by 19.8%, as was the proliferation of the large intestinal tumors which was inhibited by 41%.

The normal colonic mucosa is sensitive to the proliferative effects of hypergastrinemia involving both amidated gastrin and progastrin (Wang, et al ., 1997; Renga et al., 1997). APC1638 and *Min* mice (both have mutations in their APC gene) have an activated gastrin gene in both normal and malignant colonic mucosa, unlike the corresponding wild type mice. *Min* mice have greater proliferation levels in normal mucosa when compared to the wild type C57/BL mouse.

Hypergastrinemia induced by treatment with high daily doses of omeprazole decreases the survival of *Min* mice, which is partially reversed when co-administered with gastrin immunogen. There is an initial 2 week window when hypergastrinemia is unopposed due to lack of anti-gastrin antibodies. This effect is completely reversed when omeprazole treatment is delayed for 2 weeks allowing anti-gastrin antibody titers to rise prior to onset of hypergastrinemia.

Administration of gastrin immunogen has no effect on survival of mice of increased age (6-12 weeks). However, there is a significant effect on survival of mice immunized at an earlier age (4 weeks). Therefore, the results suggest a stage through which the mice pass and after which an anti-proliferative effect is not enough to suppress malignant progression. The subject mice thus would respond to serum-associated gastrin until onset of the gastrin autocrine pathway at a later age which may be more refractory to gastrin immunogen-induced antibodies.

When gastrin immunogen was given 2 weeks prior to omeprazole treatment a complete reversal of the survival effect of omeprazole-induced hypergastrinemia on *Min* mice was observed. This was confirmed when it was shown that the omeprazole + gastrin immunogen treated group was not significantly different from the vehicle control ($p=0.1103$).

Anti-G17 antibodies had previously been shown to be detectable in 4 week old *Min* mice by week 2 (Fig. 6) following immunization with gastrin immunogen. Determination of the anti-G17 antibody levels at the termination of the study revealed a variation in both groups as measured by specific absorbance using an ELISA assay. Some suppression of the levels of anti- G-17 antibodies in mice co-treated with omeprazole was observed perhaps due to the neutralization of the omeprazole-induced hypergastrinemia (Fig. 7, p=0.02, Mann Whitney, comparing anti-G17 (1-9):DT antibody levels in vehicle and omeprazole-treated groups).

The *Min* mouse anti-G17(1-9):DT antibody data are illustrated in Fig. 5. Measurement of the free and bound serum carboxy-amidated gastrin levels in immunized animals revealed a mean free gastrin level of 28.9pg/ml and a bound level of 36.7pg/ml (Fig. 8).

In the mice treated with gastrin immunogen + omeprazole, the free gastrin level was 45.6pg/ml (1.5 fold increase compared to animals immunized with gastrin immunogen alone, p=0.0135, mann Whitney). The bound gastrin level in the gastrin immunogen + omeprazole treated mice was 148.3pg/ml which was significantly increased when compared to animals treated with gastrin immunogen alone (2 animals had bound gastrin levels of greater than 200pg/ml) p=0.00001 when compared to bound gastrin levels in the gastrin immunogen treated group and p=0.00001 when compared to free gastrin levels in the gastrin immunogen + omeprazole group, [Mann Whitney]. The *Min* mouse serum G17 data are illustrated in Fig. 8. The data are shown in Table 1.

Table 1

Group	Mean	SD	Statistics (Mann Whitney)
1.	28.9	12.7	1vs2 p=0.191
2.	36.7	15.4	1vs3 p=0.0135
3.	45.6	19.2	2vs4 p=0.00001
4.	148.3	170.9	3vs4 p=0.00001

G17-DT immunogen administered alone induced a significant effect on survival (Fig. 9, p=0.0017). The effect on survival was greatest in the initial phase of the experiment with time to 50% survival being 9.5 weeks for the vehicle control and 14.5 weeks for

immunogen treated. G17-DT immunogen only induced this beneficial effect on survival until week 14, following which there was an exponential drop.

Example 2

As described below, the effect of omeprazole induced hypergastrinemia on the progression of the intestinal neoplasia was further studied in the *Min* (multiple intestinal neoplasia) mouse model of polyposis coli.

Confirmed Min^{+} genotype mice were randomized into 4 groups:

Group 1. OME 75mg/kg daily oral treatment

Group 2. OME + GSI 100mg oral dose/ mouse day 0 and every 3 weeks

Group 3. Oral vehicle + control immunogen

Group 4. Oral vehicle - control immunogen

Serum gastrin level was measured by RIA. Prior to end of treatment, proliferative index was determined by the bromodeoxyuridine method.

Group 1. 236pg/ml of serum gastrin

Group 4. 67pg/ml of serum gastrin

Group 1 hypergastrinemia significantly decreased survival compared to control ($p=0.0001$, log rank test) with mice in control group having a 50% survival of 16 weeks compared to 8 weeks in the omeprazole treated group. HG17-DT immunogen induced formation of serum antibodies with antigen binding capacity of > 20mg/ml resulting in effective neutralization of the hypergastrinemic state. Gastrin neutralization resulted in a reversal of the survival disadvantage induced by omeprazole ($p=0.0017$).

The hypergastrinemic mice had enhanced proliferation of normal colonic mucosa. It was found that 9.46% proliferating cells increased to 20.1%, $p<0.05$, Mann-Whitney and colonic neoplasia (22.3% increased 35.0%, $p<0.01$). Thus, the level of this experimental hypergastrinemia was in the range attained in the humans on a maintenance dose of omeprazole and resulted in enhanced progression through the adenoma:carcinoma sequence. Moreover, gastrin was confirmed as the mediator inducing a state of hyper-proliferation within both normal and neoplastic colonic epithelium. This data demonstrate the need and effectiveness for controlling hypergastrinemia on pre-malignant colon by gastrin immunogen immunization.

Polypoid carcinomas have been established *in vitro* from the large and small intestine of *Min* mice. Proliferation was assessed by a tetrazolium-based colorimetric ELISA assay. It was found that proliferation of both tumor types was not increased by amidated gastrin, but the large intestinal tumor was modestly stimulated by glycine-extended gastrin.

Gastrin immunogen immunization significantly affects the survival of *Min* mice when administered early in their life span. Moreover, the proliferation index of tumors in the large intestine was more extensively inhibited by the G17-DT immunogen than that of tumors arising in the small intestine. In this context, tumors from the large intestine of *Min* mice appear to be more sensitive to the proliferative effects of GlyG17 than tumors from the small intestine. This could be both serum-associated and tumor-associated GlyG17, the latter being due to activation of the gastrin gene in these tumors.

Immunological inhibition by G17-DT immunogen at the terminal stage of the adenoma:carcinoma sequence was not as effective. As the small intestinal tumors are the most prolific in terms of number and total tumor burden, an inhibitory effect on proliferation of less than 20% discussed above, may not be great enough to stabilize progression.

Thus, it is clear that the above-described results lead to the following conclusions:

1. The MIN mouse over-expresses the APC gene, the mutation apparently responsible for the adenoma formation a pre-cancerous stage.
2. The adenomas are sensitive to gastrin stimulation especially in early stages and/or young mice.
3. Administration of proton pump inhibitors or H_2 blockers as described, causes hypergastrinemia, hyperproliferation of adenous as and consequently shortened survival.
4. Immunization contemporaneous with omeprazole partially reverses the deleterious effect on survival. Immunization with G17-DT immunogen 2 weeks prior to the proton pump inhibitor administration resulted in complete reversal of the deleterious effects of the drug. In this regimen, the rise in antibody titers coincided with the start of the proton pump inhibitor treatment.

Treatment with the G17-DT immunogen as described is useful in reversing hyper-gastrinemic states induced by a variety of conditions, including, PA, H. pylori, atrophic gastritis, total or partial gastrectomy, treatment with proton pump inhibitors or H_2 blockers. The G17-DT immunogen is potentially effective in protecting the subject mammal, including humans, from induction of cancers responsive to gastrin (colon, stomach, pancreas, and liver).

Immunization against gastrin according to the present method of using hG17-DT induces an effective immune response in humans such that it reduces serum gastrin levels in hypergastrinemic patients to normal or lower levels.

Treatment of PA patients exhibiting hypergastrinemia with immunization (active or passive) against gastrin can be applied alone or in combination with a secondary step of anti-gastric acid administration proton pump inhibitors such as omeprazole or lansoprazole, as well as H_2 receptor blocking agents, such as rantidine cimetidine, fomatidine or nizatidine.

Example 3

Immunogens capable of inducing specific immune responses to either G17 or to G34 were prepared by standard solid state synthesis methods. Each peptide was characterized as to amino acid content and purity.

Peptides with the following amino acid sequences were synthesized:

Peptide 1 - Human G17(1-6) ("hG17(6)"): pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 2 - Human G17(1-5) ("hG17(5)"): pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 3 - Human G17(1-4) ("hG17(4)"): pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 4 - Rat G17(1-6) ("rG17(6)"): pGlu-Arg-Pro-Pro-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 5 - Human G34(1-6) ("hG34(6)"): pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 6 - Human G34(13-22) ("hG34/G17 combination"): Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Pro-Pro-Pro-Pro-Cys

Each of these peptides were conjugated to amino groups present on a carrier such as Diphtheria toxoid ("DT") via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent.

To accomplish the linkage between any of Peptides 1-6 above and the carrier, the dry peptide was dissolved in 0.1 M Sodium Phosphate Buffer, pH 8.0, with a thirty molar excess of dithiothreitol ("DTT"). The solution was stirred under a water saturated nitrogen gas atmosphere for four hours. The peptide containing reduced cysteine was separated from the other components by chromatography over a G10 Sephadex column equilibrated with 0.2 M Acetic acid. The peptide was lyophilized and stored under vacuum until used. The carrier was activated by treatment with the heterobifunctional linking agent, e.g., Epsilon-maleimidocaproic acid N-hydroxysuccinimide ester, ("EMCS"), in proportions sufficient to achieve activation of approximately 25 free amino groups per 10^5 molecular weight of carrier. In the specific instance of diphtheria toxoid, this amounted to the addition of 6.18 mg of EMCS (purity 75%) to each 20 mg of diphtheria toxoid.

Activation of diphtheria toxoid was accomplished by dissolving each 20 mg aliquot of diphtheria toxoid in 1 ml of 0.2 M Sodium Phosphate Buffer, pH 6.45. Aliquots of 6.18 mg EMCS were dissolved into 0.2 ml of Dimethyl Formamide ("DMF"). Under darkened conditions, the EMCS was added dropwise in 50 microliter ("ul") amounts to the DT with stirring. After 2 hours of incubation in darkness, the mixture was chromatographed on a G50 Sephadex column equilibrated with 0.1 M Sodium Citrate buffer, pH 6.0, containing 0.1 mM EDTA.

Fractions containing the EMCS activated diphtheria toxoid were concentrated over a PM 10 ultrafiltration membrane under conditions of darkness. The protein content of the concentrate was determined by either the Lowry or Bradford methods. The EMCS content of the carrier was determined by incubation of the activated carrier with cysteine-HCl followed by reaction with 10 mM of Elman's Reagent 5,5'dithio-bis (2-nitrobenzoic acid) 10 mM. The optical density difference between a blank tube containing cysteine-HCl and the sample tube containing cysteine-HCl and carrier was translated into EMCS group content by using the molar extinction coefficient of 13.6 x 10^3 for 5-thio-2-nitro benzoic acid at 412 nm.

The reduced cysteine content (-SH) of the peptide was also determined utilizing Elman's Reagent. Approximately 1 mg of peptide was dissolved in 1 ml of nitrogen gas saturated water and a 0.1 ml aliquot of this solution was reacted with Elman's Reagent. Utilizing the molar extinction coefficient of 5-thio-2-nitro-benzoic acid (13.6×10^3), the free cysteine -SH was calculated. An amount of peptide containing sufficient free -SH to react with each of the 25 EMCs activated amino groups on the carrier was dissolved in 0.1 M Sodium Citrate Buffer, pH 6.0, containing 0.1 mM EDTA, and added dropwise to the EMCS activated carrier under darkened conditions. After all the peptide solution had been added to the carrier, the mixture was incubated overnight in the dark under a water saturated nitrogen gas atmosphere.

The conjugate of the peptide linked to the carrier via EMCS is separated from other components of the mixture by chromatography over a G50 Sephadex column equilibrated with 0.2 M Ammonium Bicarbonate. The conjugate eluted in the column void volume is lyophilized and stored desiccated at 20°C until used.

The conjugate may be characterized as to peptide content by a number of methods known to those skilled in the art including weight gain, amino acid analysis, etc. Conjugates of these peptides and diphtheria toxoid produced by these methods were determined to have 20-25 moles of peptide per 10^5 MW of carrier and all were considered suitable as immunogens for immunization of test animals.

Example 4

Peptide hG17(1-9)-Ser9 was prepared by standard solid state synthesis methods. That peptide contains an amino terminal immunomimic of hG17 followed by a carboxy terminal spacer. This peptide comprises a 9 amino acid immunomimic of hG17 (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-) followed by the "Ser" spacer (-Ser-Ser-Pro-Pro-Pro-Pro-Cys) attached to amino acid number 9 of the hG17 immunomimic.

The peptide was conjugated to amino groups present on the Diphtheria Toxoid ("DT") immunogenic carder via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent essentially as described in Example 4.

The immunogenic constructs of this invention include an aminoterminal (1-9) G17 peptide or an aminoterminal (1-6) G34 peptide conjugated via a peptide spacer to an immunogenic carrier. The preferred G17 sequence is pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu and the preferred G34 sequence is pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys. The preferred spacer in both constructs is a Ser-peptide (Ser-Ser-Pro-Pro-Pro-Pro-Cys). The preferred immunogenic carrier is diphtheria toxoid, tetanus toxoid, keylimpet hemocyanin, and bovine serum albumin (BSA). The gastrin immunogen is defined as a conjugate of the pGlu- Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu peptide sequence, with an amino acid spacer linked to an immunogenic carrier. The preferred gastrin immunogen is defined as a conjugate of the (1-9) amino terminal (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu) peptide which is linked by peptide spacer to diphtheria toxoid.

Example 5

These experiments demonstrate that the immunogen induces antisera that bind to amidated G17 and glycine-extended G17, but not to G34. Specifically, this experiment demonstrates the gastrin-specificity of an antiserum raised by anti-G17 immunization of rabbits.

Antisera were absorbed onto a solid phase at a concentration of 100 μg/ml and displacement was determined in a competitive assay with a fixed concentration of radiolabelled G17 (100 pg/ml) and increasing concentrations of unlabelled ligands (1-25,000 pg/ml).

Figs. 11 and 12 show the displacement of [^{125}I]G17 from rabbit anti-human G17 antiserum by G17, G17-Gly and G34. The antiserum used in the test depicted in Fig. 11 was obtained form animals immunized with G17(1-9):DT and was specific for the N-terminal end of G17; the antiserum for Fig. 12 was specific for the C-terminal end of G17. G17 displaced radiolabelled G17 from both antisera preparations with a 50% inhibitory concentration (IC_{50}) of 3500 pg/ml for the rabbit anti-human G17(1-9):DT (N-terminal) and 800 pg/ml for the rabbit anti-G17 (C-terminal). Glycine-extended G17 did not displace radiolabelled G17 from the C-terminal specific antiserum, but did from the N-terminal specific antiserum (IC_{25} 12,000 pg/ml), demonstrating that the glycine-extended G17 binds to N-terminal specific antiserum,

but not to C-terminal specific antiserum. G34 displaced radiolabelled G17 from the C-terminal (IC_{25} 500 pg/ml), but not the N-terminal specific antiserum, demonstrating the specificity of the G17(1-9):DT antiserum for G17 and glycine-extended G17 and not to G34.

This invention and its preferred embodiments have been described in detail. One skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the scope of this invention.

WE CLAIM:

1. A method for treating and/or preventing hypergastrinemia comprising administering to a patient in need thereof a therapeutically effective amount of an antigastrin immunogenic composition, comprising a G17 or G34 peptide fragment or a combination thereof linked by an amino acid spacer to an immunogenic carrier.

2. The method of claim 1, wherein the spacer is a Ser peptide spacer.

3. The method of claim 1, wherein the immunogenic carrier is selected from the group consisting of diphtheria toxoid, tetanus toxoid, and keylimpet hemocyanin.

4. The method of claim 1, wherein the hypergastrinemia is associated with pernicious anemia or administration of a substance which results in increased gastrin levels.

5. A method for treating and/or preventing hypergastrinemia comprising administering to a patient in need thereof a therapeutically effectively amount of anti-G17 antibodies.

6. The method of claim 5, wherein the hypergastrinemia is associated with pernicious anemia or administration of a substance which results in increased gastrin levels.

7. A method for treating tumors associated with hypergastrinemia comprising administering to a gastrin related tumor bearing patient an anti-gastrin immunogen or anti-gastrin antibodies.

8. Use of a therapeutically effective amount of an antigastrin immunogenic composition, comprising a G17 or G34 peptide fragment or a combination thereof linked by an amino acid spacer to an immunogenic carrier in the

preparation of a medicament for treating and/or preventing hypergastrinemia in a patient.

9. Use according to claim 8, wherein the spacer is a Ser peptide spacer.

10. Use according to claim 8, wherein the immunogenic carrier is selected from the group consisting of diphtheria toxoid, tetanus toxoid, and keylimpet hemocyanin.

11. Use according to claim 8, wherein the hypergastrinemia is associated with pernicious anemia or administration of a substance which results in increased gastrin levels.

12. Use of a therapeutically effective amount of anti-G17 antibodies in the preparation of a medicament for treating and/or preventing hypergastrinemia in a patient.

13. Use according to claim 12, wherein the hypergastrinemia is associated with pernicious anemia or administration of a substance which results in increased gastrin levels.

14. Use of an anti-gastrin immunogen or anti-gastrin antibodies in the preparation of a medicament for treating tumors associated with hypergastrinemia in a gastrin related tumor bearing patient.

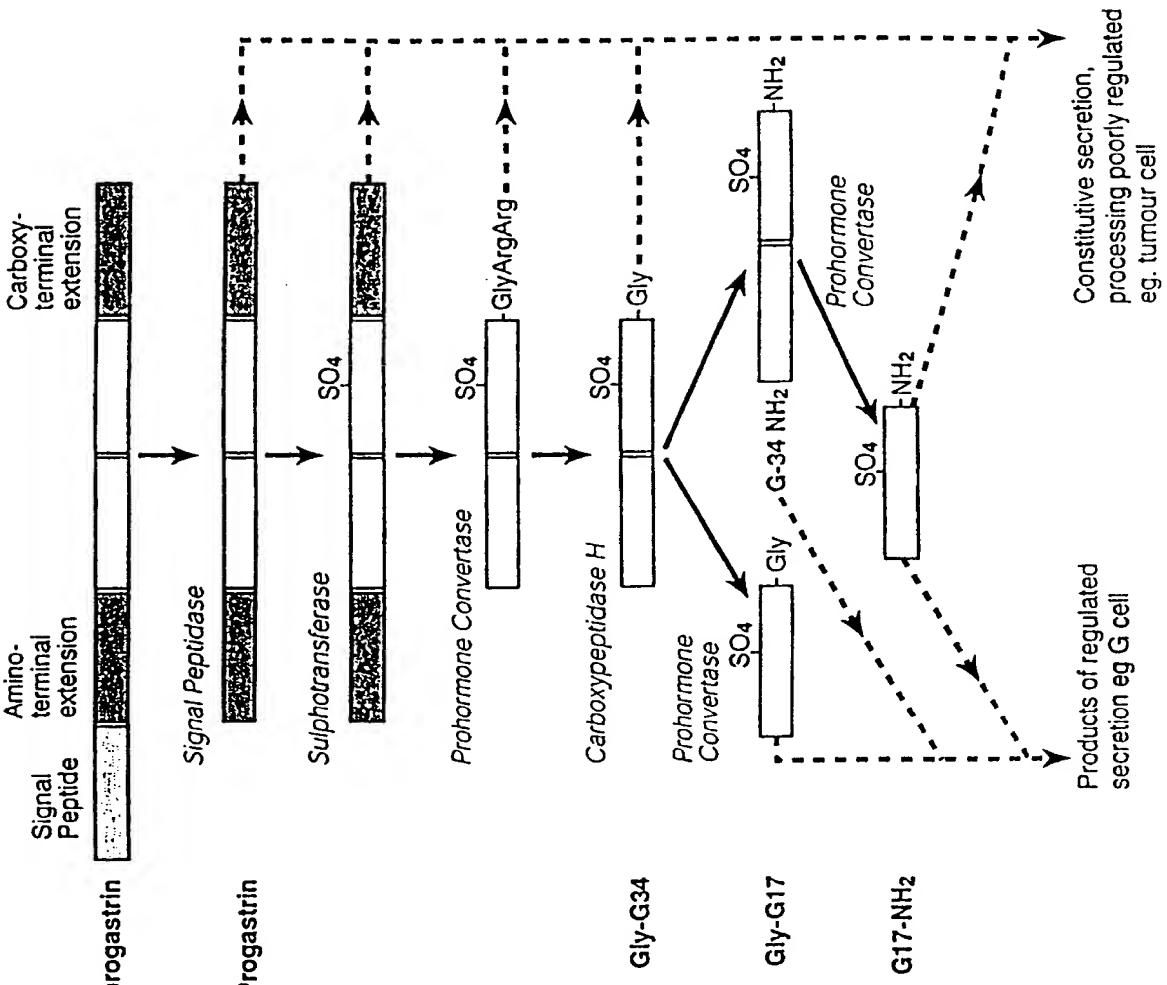
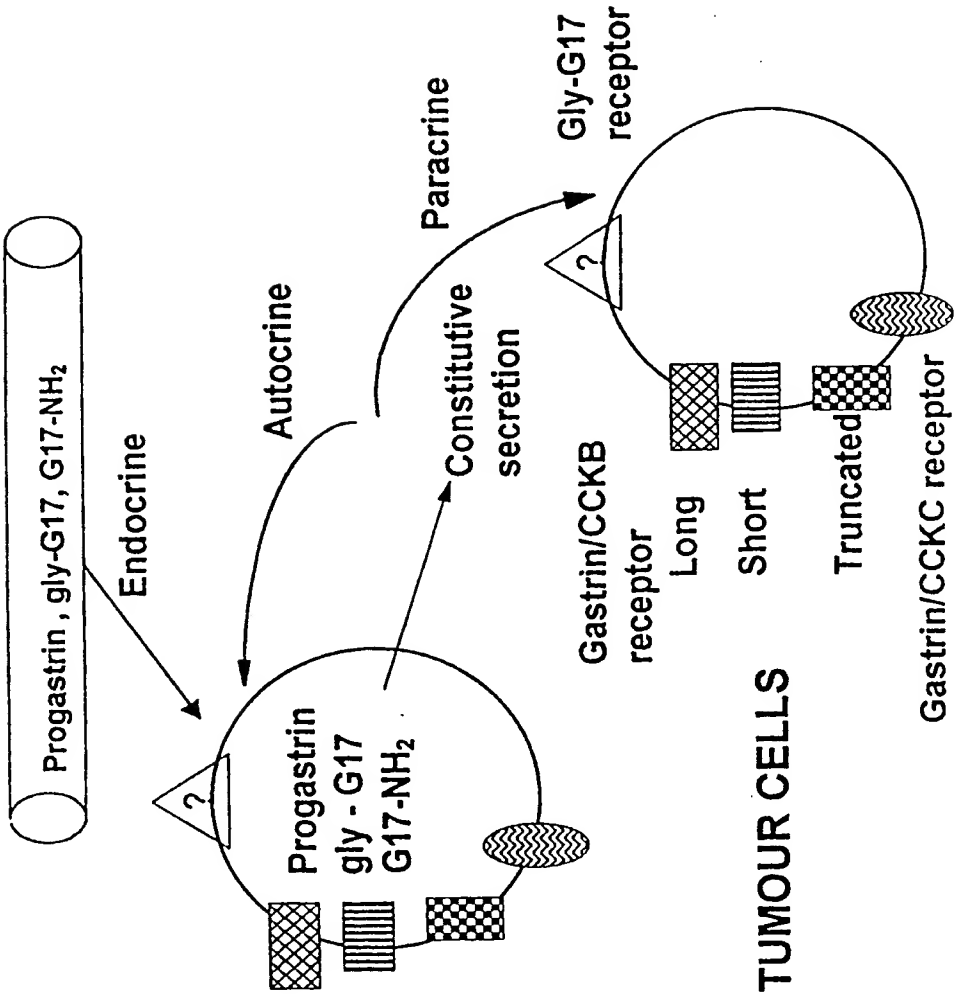

Signal Peptide
Amino-terminal extension
Carboxy-terminal extension
Preprogastrin
Signal Peptidase
Progastrin
Sulphotransferase
SO4
Prohormone Convertase
GlyArgArg
Carboxypeptidase H
Gly
Gly-G34
Prohormone Convertase
Gly-G17
G-34 NH2
Prohormone Convertase
G17-NH2
NH2
Products of regulated secretion eg G cell
Constitutive secretion, processing poorly regulated eg. tumour cell

Fig. 1


Progastrin , gly-G17, G17-NH2
Endocrine
Autocrine
Paracrine
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Gly-G17 receptor
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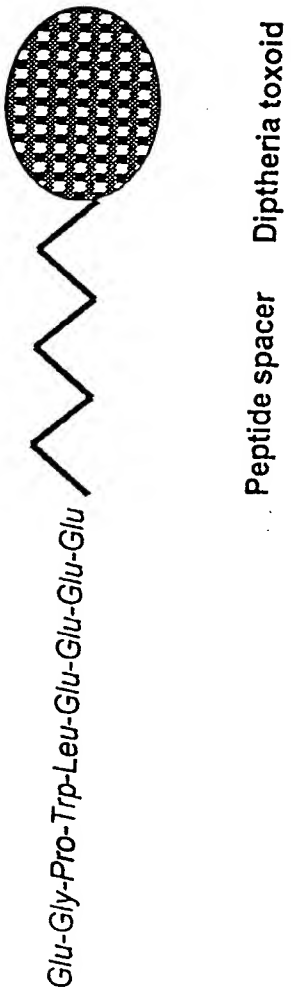
Long
Short
Truncated
Gastrin/CCKC receptor
TUMOUR CELLS

Fig. 2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Peptide spacer
Diptheria toxoid

Fig. 3

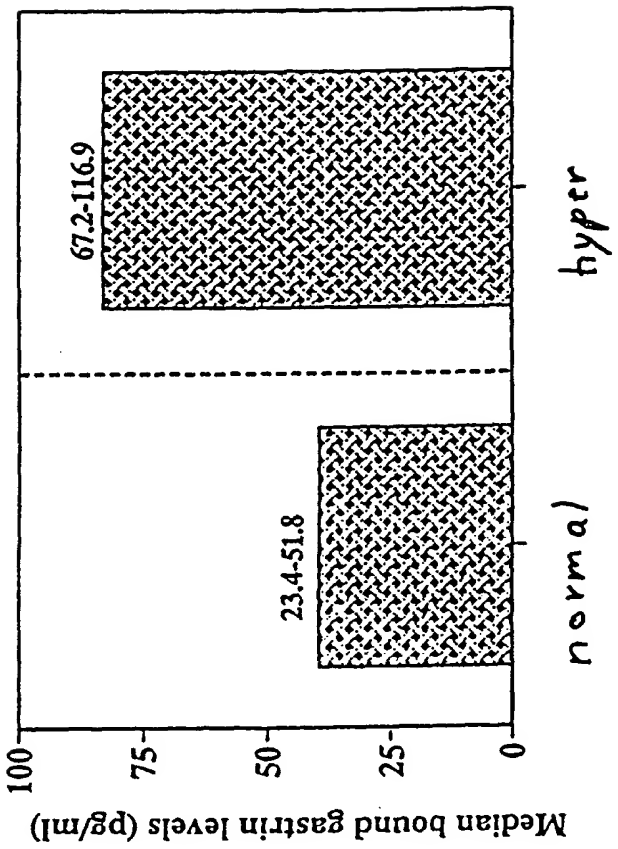

Median bound gastrin levels (pg/ml)
100
75
50
25
0
67.2-116.9
23.4-51.8
hyper
normal

Fig. 4

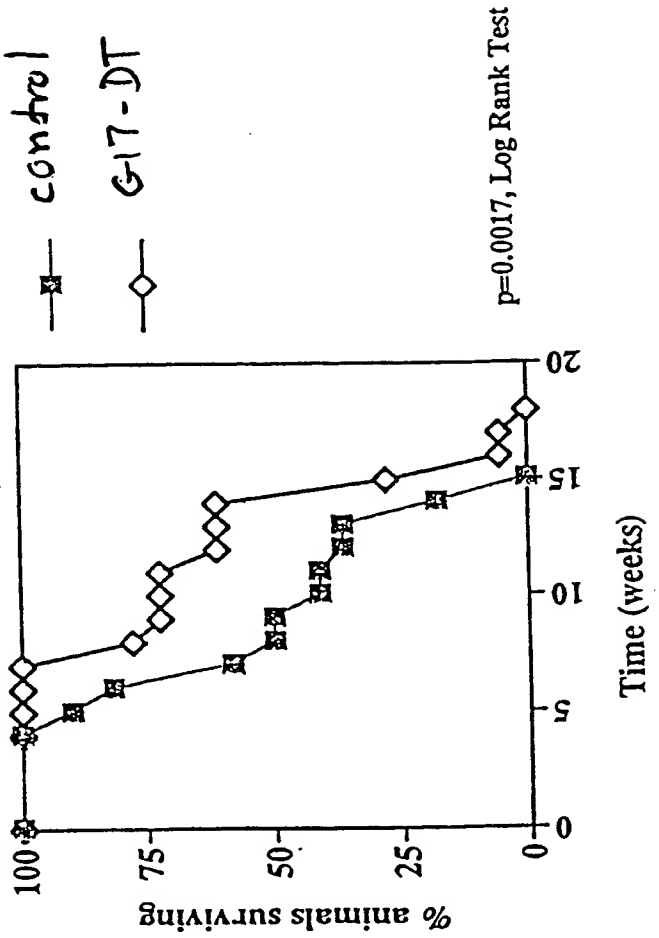

control
G17-DT
p=0.0017, Log Rank Test
% animals surviving
100
75
50
25
0
0
5
10
15
20
Time (weeks)

Fig. 5

Fig. 6

MEAN PROLIFERATION INDEX (%)

Group	Small Intestine		Large Intestine	
	Crypts	Tumour	Crypts	Tumour
Vehicle	6.45 (1.9)	33.3 (6.6)	6.05 (2.7)	43.2 (10.5)
G17-DT	5.87 (0.8)	26.7 (5.1) $p<0.005$	5.80 (2.5)	25.6 (7.6) $p<0.0000$

Fig. 7

Timed anti - G17(1-9):DT antibody in Min mice

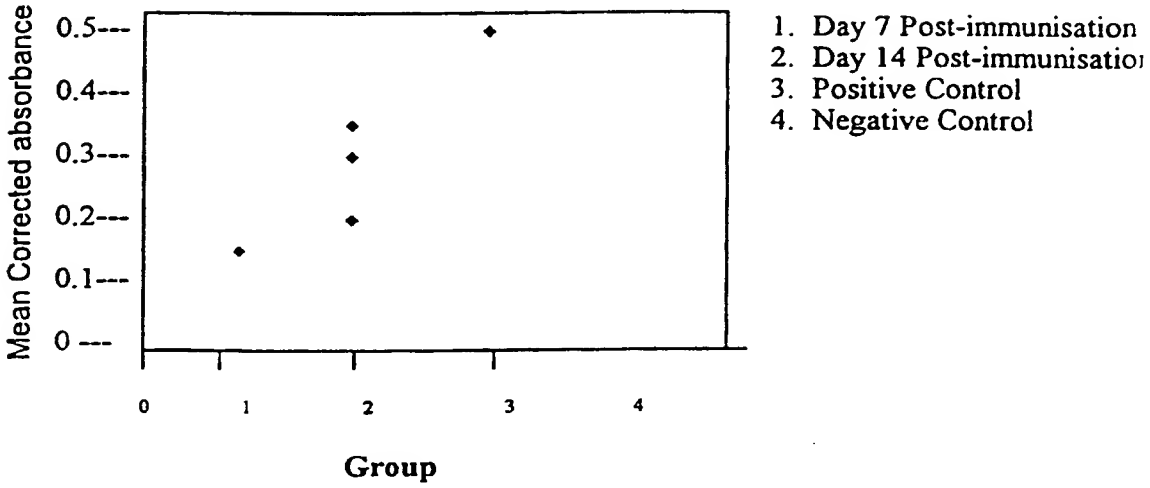
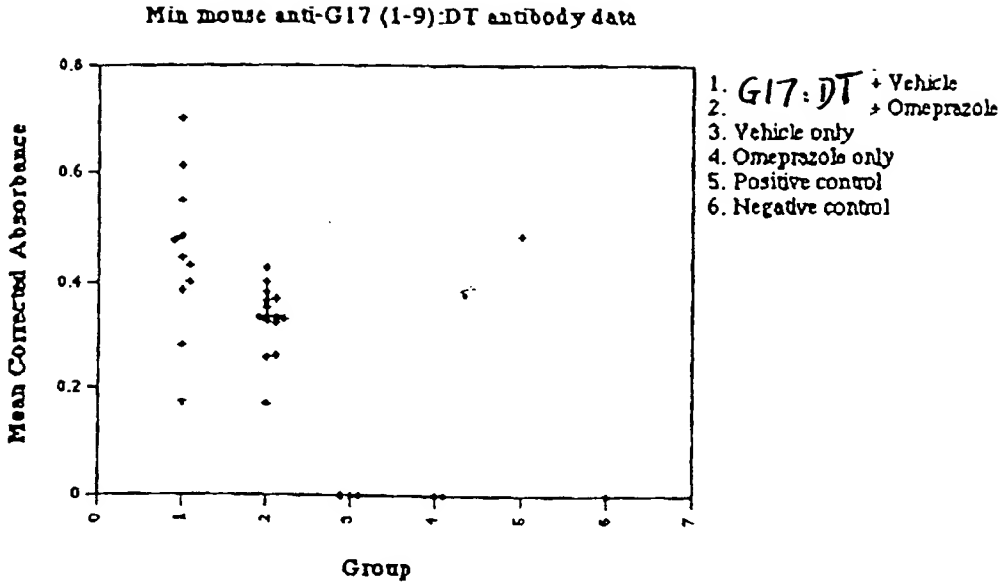


Fig. 8



Group 1 versus Group 2, p=0.02, Mann Whitney

Fig. 9

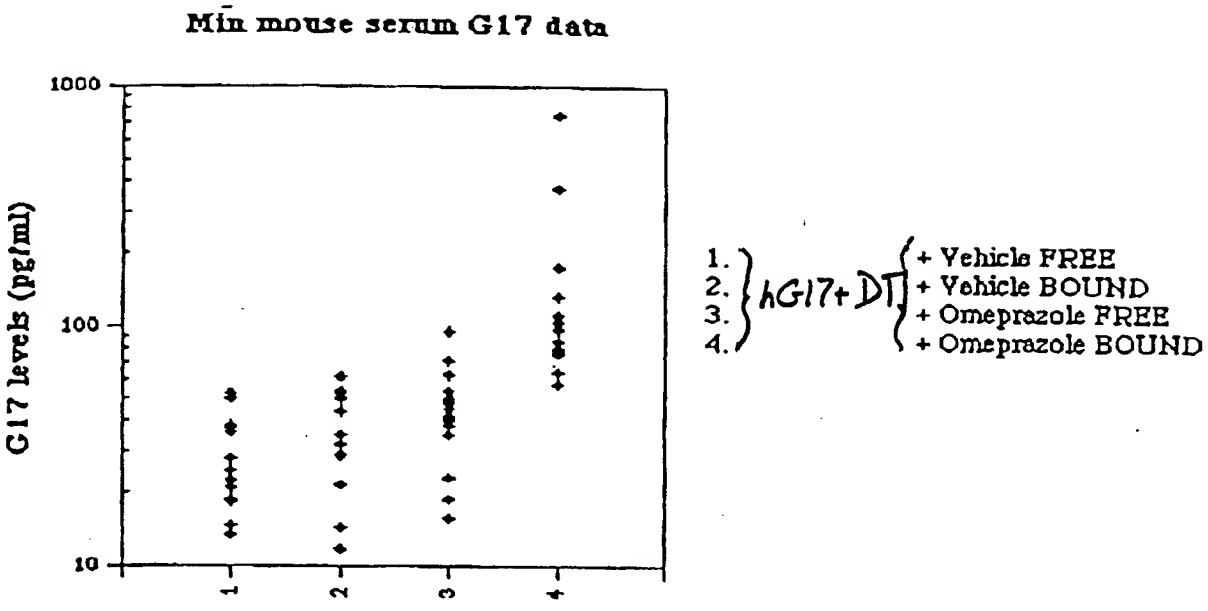

Min mouse serum G17 data
G17 levels (pg/ml)
1000
100
10
1
2
3
4
1.
2.
3.
4.
hG17+DT
+ Vehicle FREE
+ Vehicle BOUND
+ Omeprazole FREE
+ Omeprazole BOUND

Fig. 10

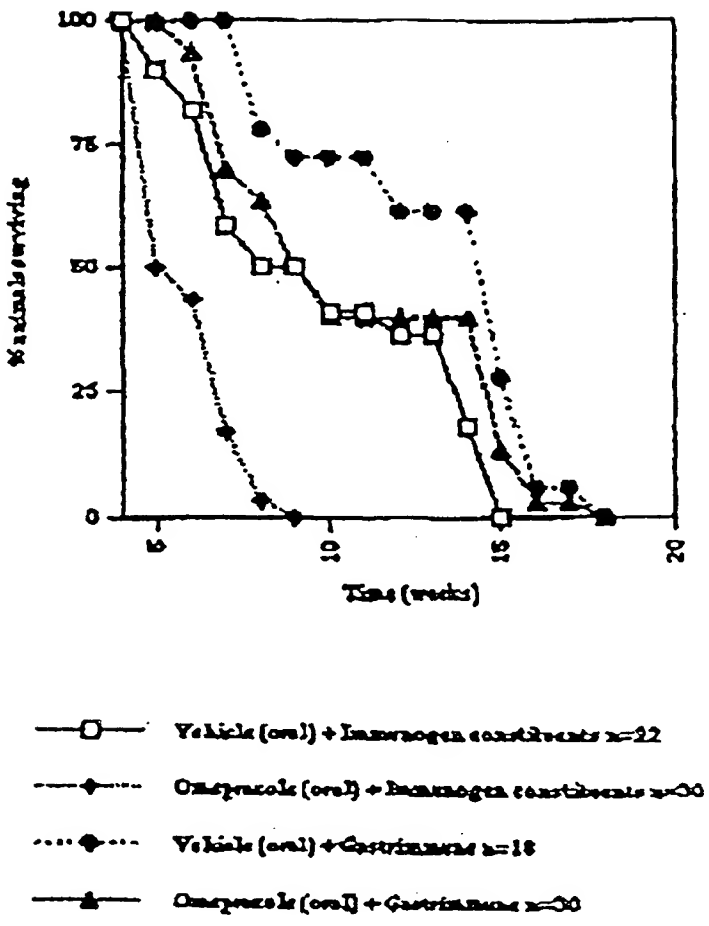


FIG. 11

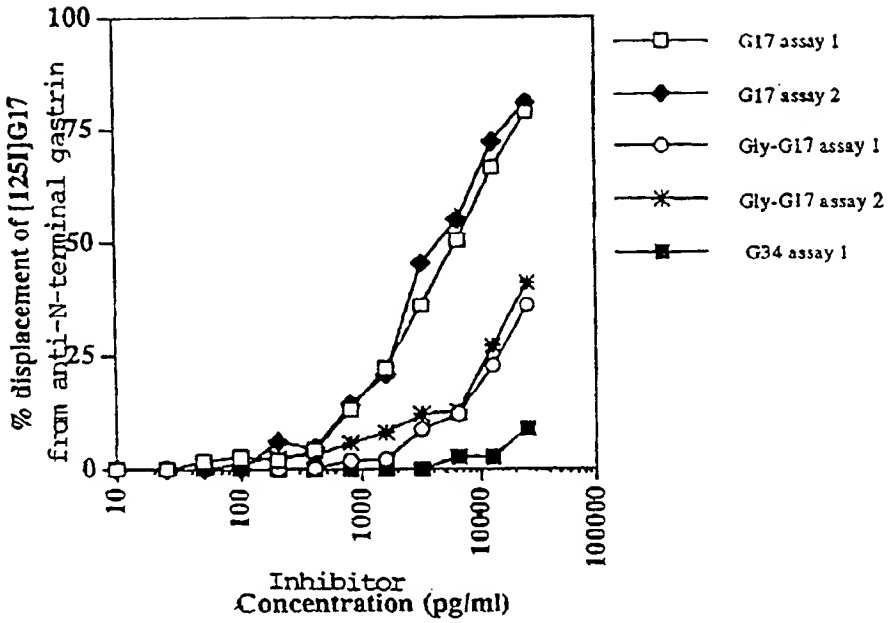
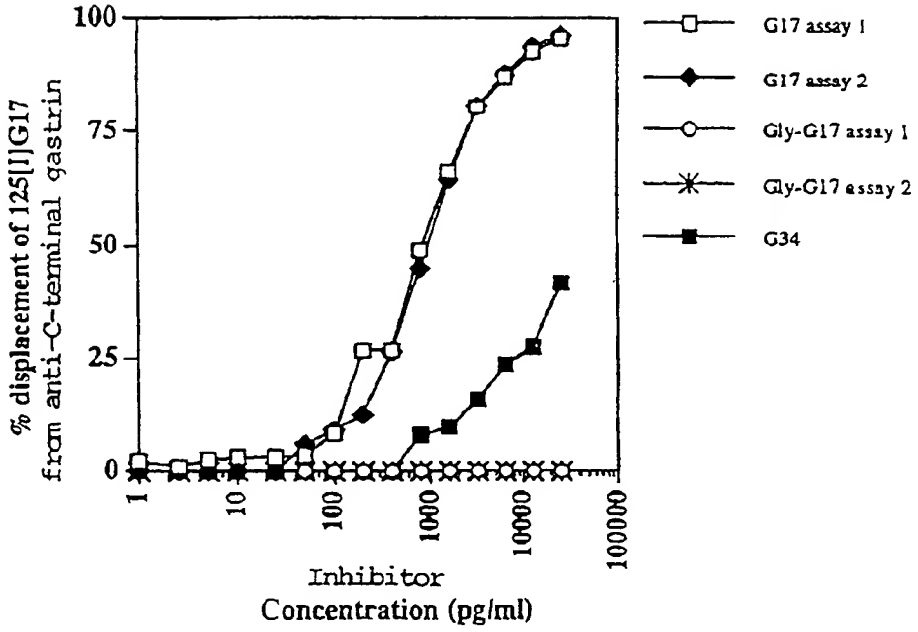

100
75
50
25
0
% displacement of [125I]G17 from anti-N-terminal gastrin
10
100
1000
10000
100000
Inhibitor Concentration (pg/ml)
G17 assay 1
G17 assay 2
Gly-G17 assay 1
Gly-G17 assay 2
G34 assay 1

FIG. 12


100
75
50
25
0
% displacement of 125[I]G17 from anti-C-terminal gastrin
1
10
100
1000
10000
100000
Inhibitor Concentration (pg/ml)
G17 assay 1
G17 assay 2
Gly-G17 assay 1
Gly-G17 assay 2
G34

SEQUENCE LISTING

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<120> Prevention and Treatment of Hypergastrinemia

<130> 1102865-0035

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/385; CO7 K 7/06, 08
US CL : 424/184.1, 194.1, 198.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 194.1, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN on line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THORNDYKE M., Identification and localozation of material with gastrin-like immunoreactivity in the neutral ganglion of a photochordate, Ciona intestinalis. Regulatory Peptides, Vol. 16, pages 269-279, 1986, see abstract	1-14
X --- Y	WATSON et al. Gastrimmune Raises Antibodies that Neutralize Amidated and Glycine extended Gastrin-17 and inhibit the growth of colon cancer. Cancer Research, 1996, Vol.56, pages 880-885. See abstract, Fig.2, p. 883, right column.	1,3-5,7,10-12,14 ----- 2,9,6,13
X --- Y	WATSON et al. Anti-gastrin antibodies raised by gastriimmune inhibit growth of the human colorectal tumor AP5. Int. J. Cancer, 1995, Vol. 61, pages 233-240; see abstract	1;3-5, 7, 10-12, 14 ----- 2,9,6,13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search 19 AUGUST 1999	Date of mailing of the international search report 19 OCT 1999
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,023,077 A (GEVAS et al) 11 June 1991, see abstract, claims 1-8.	1-14
X	US 5,468,494 A (GEVAS et al) 21 November 1995, see abstract, claims 1-6.	1-14
X	US 5,609,870 A (GEVAS et al) 11 March 1997, see abstract, claims 1-5.	1-14
X	US 5,607,676 A (GEVAS et al) 04 March 1997, see abstract, claims 1-13.	1-14
X	US 5,622,702 A (GEVAS et al) 22 April 1997, see abstract, claims 1-4.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10751

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional search fees must be paid.

Group I, claims 1-4, 8-11, and claims 7, 14 in part, drawn to methods of use of G17 or G34 peptide coupled to immunogenic carrier.

Group II, claims 5,6, 12, 13, and claims 7, 14 in part, drawn methods of use of anti-G17 antibodies.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I, II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they do not have a the same or corresponding special technical features. The method of Group I employs G17 or G34 peptide conjugated to immunogenic carrier via a peptide link, whereas the method of Group II utilizes anti-G17 antibody. Therefore, the lack of unity is present because the linking technical feature is not a "special technical feature" as defined by PCT Rule 13.2.

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(54) Title: METHOD FOR THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE

(57) Abstract

A method for the treatment of gastroesophageal reflux disease comprising a combination of active immunization with an anti-gastrin immunogenic composition with an antagonist which blocks or inhibits the gastric acid pump activity; or alternatively administering purified anti-gastrin antibodies with a H_2 antagonist or proton pump inhibitor of the gastric acid producing enzyme system.

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METHOD FOR THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE

The present invention relates to a combination of immunological, antihormonal and enzyme inhibitory methods for the treatment of gastroesophageal reflux disease.

BACKGROUND OF INVENTION

Gastroesophageal reflux disease ("GERD") is a common and chronic disorder which requires long-term, even lifelong, therapy. GERD is commonly known as heartburn, and is characterized by a retrosternal burning sensation, and regurgitation of the stomach contents. About 40% of adults in the United States have experienced occurrences of the disease, and approximately 10% have daily troubling symptoms.

GERD occurs when there is an abnormally prolonged contact time between the esophageal mucosa and refluxate, which is believed to be primarily gastric acid (DeVault, et al., *Mayo Clinic Proc.* 69:867-876, 1994 and Redmond, et al. In "Gastroesophageal Reflux Disease" Ronald Hinder ed., R.G. Landes Co., Ch. 1, pages 1-6, 1993). The regurgitation of the gastric contents and duodenal juice is believed to be due to either an incompetent lower esophageal sphincter or more frequently to an inappropriate sphincter relaxation at the time of transfer of the stomach contents between stomach and small intestine. The resultant reflux of acid and other materials from the stomach may induce pain or damage the esophageal mucosa. This damage to the esophageal mucosa may lead to esophagitis which is characterized by inflammation of the esophageal mucosa, bleeding, cytological changes, peptic esophageal stricture, esophageal ulcer and Barrett's metaplasia, depending on the severity of the disease.

Gastric acid is produced by parietal cells in the stomach upon stimulation by acetylcholine, histamine and gastrin following the binding of each of these compounds with specific receptors on the surface of the cells. The peptide hormone gastrin is produced by mucosal cells in the stomach. Gastrin is secreted into the blood stream, and is the most potent stimulant of acid secretion by the parietal cell. Gastrin is present in two molecular forms, heptadecagastrin (G17) and tetratriacontagastrin (G34). G17 is the primary stimulator of meal-induced gastric acid

secretion and is 1500 times more potent than histamine, accounting for 60% of the gastrin-mediated acid release. It has also been found that in GERD patients having an abnormal sphincter, the postprandial levels of gastrin are twice those of a normal person and remain high, beyond 3 hours after the meal (Wetscher, et al. In *Gastroesophageal Reflux Disease*, R.A. Heinder, ed. R.G. Landes Co., Ch. 2, pages 7-29, 1993).

Normal esophageal pH is greater than pH 4. The acid refluxate from the stomach lowers the pH value of the esophagus to less than 4, which results in damage to the esophageal mucosa and the development of GERD. In normal individuals, acidic refluxate is cleared by elimination of the refluxate by peristalsis of the esophagus and by neutralization of the acid with the bicarbonate produced by submucosal esophageal glands, and the bicarbonate present in swallowed saliva. In GERD patients, these mechanisms of acid neutralization are not sufficient to restore the normal esophageal pH values and prevent mucosal damage, since reflux of stomach contents occurs more frequently, and for a more prolonged period of time, than in normal individuals (Booth, et al., *Arch. Surg.* 96: 731-734, 1968 and Demeester, at al., *Ann. Surg.* 184: 459-470, 1976). Since it is not medically practical to alter the esophageal acid neutralization mechanisms, GERD therapies are directed to raising the pH of the stomach contents.

Currently, various therapies are available for the treatment of GERD. Historically, the medical treatment for GERD consisted of using antacids as acid neutralizing agents or anti-refluxants, such as alginates, for alleviating an acute onset of the disease. However, these treatments are not effective for the therapy of chronic and severe symptoms of GERD. Systemic medications currently used for treating GERD include the histamine receptor antagonists, cimetidine and ranitidine, which are acid-suppressive agents directed to the inhibition of the four histamine type 2 ("H_2") receptors. These agents prevent the normal binding of histamine, thereby inhibiting the parietal cell from secreting gastric acid and thus, they increase the pH of the stomach contents. The most commonly used histamine H_2 - antagonists are cimetidine hydrochloride (Tagamet®, SmithKline Beecham Pharmaceuticals), ranitidine hydrochloride (Zantac®, Glaxo Pharmaceuticals), fomatidine (Pepcid®, Merck & Co.) and nizatidine (Eli Lilly & Co.). The use of these H_2 antagonists is the standard

treatment of acid-caused peptic disorders including GERD, since surgery, a more radical approach, is usually contraindicated.

Despite the widespread acceptance of histamine H_2 receptor blockers, controlled studies on GERD patients treated with these acid inhibiting compounds have yielded variable results on the healing of esophagitis and persistent symptomatic responses, such as continued acid production in the stomach. Studies using cimetidine and ranitidine in GERD patients, at doses and durations that had been proven effective in healing peptic ulcers, were not effective in GERD (Sabesin et al. *Arch. Intern. Med.* 151:2394, 1991). At higher doses and duration of the H_2-antagonist therapy (400-800mg and 150-300mg twice daily, respectively, for cimetidine and ranitidine), approximately 50-70% (mean, 61%) of patients had symptomatic relief of GERD, and 0 to 82% (mean, 48%) had healing of their esophagitis as endoscopically determined (DeVault, et at. *ibid*; Koelz, H.R. Scand. J. Gastroenterol. 24:25-26, 1989 and Fennerty and Sampliner, *Arch. Intern. Med.* 151:2365-2366, 1991).

The healing of ulcerated or eroded esophageal mucosa requires a longer and more profound acid suppression than is necessary in treating other gastrointestinal ulcers. In patients in whom the symptoms of GERD disappeared after an effective treatment with histamine blockers, the symptoms of the disease reappeared soon after the treatment was discontinued (Antonson, et al. *Gastroenterology* 98: A16, 1990 and Bardhan, et al. *Gastroenterology* 98: A18, 1990).

Many patients with severe GERD hypersecrete gastric acid and may require high doses of H_2 antagonists, which become problematic in terms of patient compliance and long term use of these agents. The high doses of H_2 blockers when given to patients for a long period of time may cause undesirable side effects such as, blood pressure and heart problems. The increase in the effective dosage required to bring about relief of GERD symptoms results in very costly therapy. Although treatments of esophagitis vary widely depending on the severity of the disease, the more severe, high-grade types of the disease respond poorly to standard doses of histamine blockers. Approximately 50% or more of patients with GERD do not respond to histamine H_2 antagonist therapy and still require some other form of treatment. In addition, the effective treatment of GERD not only depends on increasing the concentration of histamine blockers or the hydrogen pump inhibitors

which have also been found to be effective in the treatment of GERD, but effective dosaging must be frequent, since the compounds have limited transient time in the patient and must be given in some situations approximately 4 times daily. In a significant number of cases, the patient is not responsive to H_2 blockers.

Proton pump inhibitors omeprazole (Astra AB), or anti-H^+/K^+-ATPase enzyme inhibitory compound, as well as its analogue, lansoprazole, (Takeda Chemicals) or pantoprazole (Byk Gulden) which inhibit acid secretion in the stomach by inhibiting the proton (hydronium ion) pump mechanism for producing hydrochloric acid in the parietal cell, have been found to be more effective than histamine H_2 blockers in alleviating the symptoms of GERD esophagitis. The resulting increase in pH induced by omeprazole leads to approximately 62-94% (mean, 83%) in symptomatic relief and a healing of the esophagitis occurs in 71-96% (mean, 78%) of the patients in 4-8 weeks of treatment for GERD, almost twice that of ranitidine (DeVault et al. *ibid*; Zeitoun, P. *Scand. J. Gastroenterology* 166 (Suppl.): 83, 1989). A disadvantage of using omeprazole, lansoprazole, or pantoprazole similar to the case with histamine blockers, is that the compound must be administered at higher doses (20 mg, twice daily or 40 mg once daily) than the dosages required to treat gastric and duodenal ulcers (20mg, once daily), and for a longer period of time in order to effectively treat GERD.

Furthermore, the prolonged use of histamine blockers or omeprazole for the treatment of GERD results in an increase in serum gastrin levels (2 to 4 times the basal rate). It has been suggested that the increase in gastrin levels could lead to undesirable side effects such as dangerous trophic effects on the human gastric mucosa (Festen, et al. *Gastroenterology* 87: 1030-1034, 1984; Jansen, et al. *Gastroenterology* 99: 621-628, 1990 and Sontag, et al. *Gastroenterology* 102-109, 1992).

Co-assigned U.S. Patents No. 5,023,077 and No. 5,609, 870 disclose immunogenic compositions useful for controlling gastrin levels in a patient by generating anti-gastrin antibodies. Thus U.S. Patent No. 5,023,077 and No. 5,609,870 disclose that the immunogenic compositions are useful for the treatment of gastric and duodenal ulcers and gastrin induced or responsive cancers and the disclosures are hereby incorporated entirely by reference into the present description.

There remains a need in the art for additional methods and compositions for the successful therapy of GERD.

SUMMARY OF THE INVENTION

The invention combines a method for reducing gastric acid in the stomach by inhibiting the enzyme responsible for gastric acid production or secretion of gastric acid and an immunological method for reducing or preventing the increase of circulating gastrin. It is the object of the present invention to use anti-gastrin immunogenic compositions in the therapy of GERD in combination with administering effective doses of a proton pump inhibitor or H_2 antagonist so as to substantially raise the gastric pH while preventing elevated levels of circulating blood gastrin hormone.

This invention is directed to the treatment of GERD by gastric acid suppression by administration of a proton pump inhibitor or H_2 blocker together with the immunological reduction of circulating gastric hormone by neutralization of heptadecagastrin G17 or tetratriacontagastrin G34, or both G17 and G34 either by administration of exogenous specific antibodies or *in situ* by an immunogenic composition against gastrin.

It is the preferred embodiment of the invention to treat a patient with GERD by administering effective omeprazole dosages with effective dosages of antigastrin G17 antibodies.

It is the more preferred embodiment to keep the frequency of anti-gastrin immunogen parenteral administration to a patient suffering from GERD at a single effective dose or at least at only a few doses thereof.

Yet another preferred method of the invention is to pre-treat the GERD patient with gastrin immunogen or anti-G17 antibodies before administering the gastric acid producing enzyme inhibitor (i.e. proton pump inhibiting compound).

In one embodiment, the invention concerns a combination therapy with a histamine H_2 antagonist, such as ranitidine, cimetidine, fomatidine or nizatidine, or a proton pump inhibitor such as, omeprazole or lansoprazole, using standard dosing procedures for H_2 antagonist or proton pump inhibitor, respectively, as described by the art. In the preferred combination therapy, a patient is actively immunized with an immunological composition comprising gastrin 17(1-9)- h(G17)ser9-Diphtheria Toxin

(see U.S. Patent Nos. 5,023,077 and 5,468,494 (co-assigned). Once the patient is immunized, histamine H_2 antagonist or proton pump inhibitor therapy is administered for 2-12 weeks or until the desired serum anti-gastrin 17 antibody titer is reached. The novel combination therapy provides a more effective method for controlling acid output by the stomach, since acid production is thus controlled by two independent mechanisms, which results in a more effective method for treating GERD, including the more severe cases of the disease. In addition, the therapy would be a less costly method for treating GERD, without the problems with patient compliance associated with long term standard therapies. Furthermore, the high gastrin levels associated with standard therapies, particularly with omeprazole, are neutralized, and thus, the undesirable side effects are reduced.

The method of this invention for treating GERD permits a reduced dosage of the acid reducing agent both at the acid producing level as well as the acid production stimulating level (gastrin). This reduction of dosages is desirable in the usually prolonged treatment.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 illustrates experimental data concerning the percentage of time that the gastric contents remain above pH 3 in different groups of pigs treated with ranitidine (4 animals), omeprazole (5 animals) and hG17(1-9) (4 animals), as compared to six (6) control animals.

FIGURE 2 illustrates the percentage of time that the gastric contents remain above pH 4 in a group of untreated (control) pigs (5) and groups of pigs treated with human gastrin 17(1- 9)Ser9-Diphtheria Toxin (4 animals), ranitidine (4 animals) and omeprazole (5 animals) as described in **Figure 1.**

FIGURE 3 depicts the baseline median pH of the gastric contents of a group of six (6) untreated (control) pigs and groups of four (4) pigs treated with human G17(1-9)Ser9-Diphtheria Toxoid, three (3) pigs with ranitidine and five (5) pigs with omeprazole as described in **Figure 1**.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel combination of methods for the treatment of gastroesophageal reflux disease. The combined method on the one hand comprises inhibiting the normal binding of the hormone gastrin 17 to its physiological receptor by actively immunizing the patient against his or her own gastrin 17 hormone. Alternatively or additionally, the hormone gastrin 34 can be neutralized by active or passive immunization with G34 or C-terminal G17 peptide fragment. On the other hand, the method provides inhibition of production of gastric acid either by proton pump inhibition or H_2 receptor blockage.

The invention provides a novel immunological method for the treatment of gastroesophageal reflux disease using a peptide immunogen which raises sufficient gastrin 17 or gastrin 34 antibody levels in a patient so as to affect the binding of the gastrin 17 or gastrin 34 to its physiological receptors in the patient and raise the pH of the stomach. Gastric acid secretion in the stomach can thus be controlled. The pH of the stomach contents is simultaneously raised to a sufficient pH level, e.g., greater than pH 3 for a prolonged and sufficient period of time to alleviate the GERD symptoms and heal the acid-induced esophagitis. According to the invention, anti-G17 antibodies are induced in the patient by active immunization with peptide immunogens which comprise a G17 immunogen conjugated to an immunogenic carrier. The antibodies raised in the patient by the immunogens selectively and specifically bind gastrin hormone G17 or G34 or both, and neutralize and inhibit separately or together the normal binding of gastrin G17 or G34 or both to its receptors in the parietal cells, thereby controlling acid output in the stomach and preventing gastric acid damage of the esophageal mucosa during regurgitation.

A preferred embodiment of the inventive method provides a single administration of an active gastrin 17 or G34 immunogen, which has several advantages over the standard therapies of the art for treating GERD in that problems with patient compliance and undesirable side effects as a result of the therapy are eliminated. Other advantages of using the immunological methods for the treatment of GERD include the use of a limited number of administrations. A single primary administration with appropriately spaced boosters may last for approximately 6 months to a year. Another advantage is that, in a combination therapy with H_2 agonists or

proton pump inhibitors, effective anti-gastrin 17 antibody titers can be maintained by occasional booster shots while the gastric acid inhibitor dosing is reduced or discontinued. Another advantage of this invention is that the maintenance of antigastrin antibody titers reduces or prevents excessive levels of gastrin in hypogastrinemia which would otherwise result from administration of a proton pump inhibitor or H_2 blocker. A booster shot of the immunological composition prolongs anti-gastrin 17 immunity and gastric acid suppression. Still another advantage of this method is that the immunization allows a sufficient time for the esophagitis to completely heal. Additionally, no surgery is required. Yet another advantage is that combination therapy is more useful for treating severe cases of GERD, without causing undesirable side effects, since excess serum gastrin 17 peptides are physiologically neutralized. In patients where the GERD condition is alleviated, discontinuation of the booster dose may result in resumption of normal gastrin levels.

According to the method of the invention, an immunogen is prepared using peptides or chemical structures that mimic the amino terminal end of gastrin 17 or of gastrin 34. The immunogens and immunogenic compositions of the invention are those described in U.S. Patent No. 5,023,077, U.S. Patent No. 5,469,494 and U.S. Patent No. 5,609,870. The disclosures of these issued patents are hereby incorporated by reference in their entirety. U.S. Patent Nos. 5,023,077, 5,469,494, and 5,609,870 disclose compositions containing anti-gastrin 17 immunogens as well as anti-gastrin 34 immunogens and methods of using these compositions for the treatment of gastric and duodenal ulcers and gastrin responsive cancers.

In the present invention, effective dosages ranging from 0.1mg to 5g of the immunogenic composition are administered for the treatment of GERD combined with 10-80mg daily dose of omeprazole. An effective dosage of the immunogenic composition is capable of eliciting an immune response in a patient and inducing antibody titer against human gastrin 17 within 1-3 months after immunization.

Effective treatment of GERD according to this method results in maintenance of the pH of the stomach contents above pH 3 or 4, and for a more prolonged period of time than with H_2 antagonist therapy. Maintenance of the stomach pH above 3 or 4 is essential in the treatment of GERD, since refluxate material having a pH below 2.0 causes esophagitis by protein denaturation and cell

damage, and pH values below 2.5 triggers painful episodes in a patient. When the pH is maintained above 2.5, pain perception is almost nonexistent (Smith, et al *Gastroenterology* 96: 683-689, 1989) and damage to the esophageal wall is minimized.

The immunogens and immunogenic compositions of the invention typically induce specific antibody responses after a single administration. However, it may take several weeks or months for antibody titers to rise to the desired levels effective for the treatment of GERD.

Combination therapy with a histamine H_2 antagonist, such as ranitidine, cimetidine, fomatidine and nazatidine, or a proton pump inhibitor, such as omeprazole or lansoprazole, is designed so that a GERD patient is immunized with an immunogenic composition of the invention, and administration of H_2 antagonist is provided on a daily basis, at least once a day for the first 2-12 weeks of treatment or until the desired serum level of anti-gastrin 17 antibodies is obtained.

Desired anti-gastrin 17 serum levels range from 10 to 300 pmole/ml. Once the desired serum levels of anti-gastrin 17 antibody titer are obtained as measured by ELISA or RIA, the non-immunological gastric acid inhibiting drug portion of the combination therapy may be reduced or discontinued.

In the following Examples, the anti G17 immunogenic composition, 150mg ranitidine and 60mg omeprazole were administered to pigs and the resulting changes in the pH of the stomach contents before and after treatment were measured. Specifically, following the stomach pH measurements of the untreated control state of each pig, the stomach pH of the same pigs was measured after the animals were treated with either ranitidine, or composition of human gastrin 17(1-9)-h(G17)ser9-Diphtheria Toxoid (Gastrimmune), or omeprazole administered individually and at different times in each of four animals (pigs).

Example 1

Gastrin neutralization was achieved by using the immunological composition Gastrimmune which is composed of the amino terminal domain of gastrin-17 linked, via an amino acid or peptide spacer to diphtheria toxoid which acts as the immunogenic carries. The antibodies raised by virtue of the design of the immunogen, cross-reacted with both amidated and glycine-extended gastrin-17, two known

proliferative forms of gastrin.

Serum antibody titers rose within 2 weeks of the initial immunization to levels with an antigen binding capacity of > 10^{-9}M. The presence of anti-gastrin antibodies within the serum of Gastrimmune-immunized mice was confirmed by using an ELISA. As expected, no bound gastrin levels were detected in animals immunized with control immunogen.

Example 2

As can be seen in **FIG. 1** and **FIG. 2**, the pH of the stomach contents remained above pH 3 or 4 in anti-gastrin 17 immunized pigs for a longer period of time than in the pigs treated with ranitidine. In omeprazole treated pigs the stomach pH was maintained above pH 3 or 4 for a longer period of time than pigs which were treated with ranitidine and anti-G17 immunized pigs.

In addition, **FIG. 3** shows the median pH exhibited by the stomach contents of control pigs when compared to ranitidine, anti-G17 immunization and omeprazole treatment. The data shows that the stomach pH is maintained at higher levels in pigs than those treated with ranitidine or anti-G17 immunization therapy. Anti-G17 immunized pigs had a median pH higher than ranitidine treated pigs.

Treatment of the pigs with ranitidine was less effective in preventing acid output from the stomach. Omeprazole treatment highly inhibited acid output. A single administration of anti-gastrin 17 immunization inhibited stomach acid output at a level of effectiveness between ranitidine and omeprazole, and sufficient to reduce the stomach acid output levels and increase the stomach pH for the effective treatment of GERD.

A treatment which combines the gastric acid secretion with proton pump inhibitors or H_2 histamine blockers with the novel immunization by e.g. Gastrimmune can thus result in maintaining favorably raised pH in the stomach. Furthermore, the treatment with occasional, effective boosters of the antigastrin immunogenic composition can eventually, possibly within a few months, obviate any additional treatment with the anti-acid secretion drugs, such as e.g. omeprazole or ranitidine.

One of the possible advantages of the administration of a proton pump

inhibitor or H_2 blocker after immunization with an antigastrin immunogen, as described, resides in the use of lower amounts of the proton pump inhibitor or H_2 blocker for effective lowering of gastrin acid secretion or raising of stomach pH to about 3-4.

Example 3

The human patient suffering from GERD is immunized with 200 μg - 400 μg of primary i.v. inoculation of G17 (1-9) Ser : DT immunogen composition. After 2 weeks a booster of 100 - 200 μg of the G17 (1-9) Ser : DT composition is similarly administered. When the anti-G17 titer has reached a level of about 10-300 pmole/ml sufficient to lower the serum gastrin level to near normal with a concomitant lowering of gastric acid secretion, about 10-20 mg oral omeprazole preparation is administered daily to further reduce or stabilize the gastric secretion at a level which essentially eliminates or substantially ameliorates the GERD symptoms.

Example 4

Immunogens capable of inducing specific immune responses to either G17 or to G34 were prepared by standard solid state synthesis methods. Each peptide was characterized as to amino acid content and purity.

Peptides with the following amino acid sequences were synthesized:

Peptide 1 - Human G17(1-6) ("hG17(6)"): pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 2 - Human G17(1-5) ("hG17(5)"): pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 3 - Human G17(1-4) ("hG17(4)"): pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 4 - Rat G17(1-6) ("rG17(6)"): pGlu-Arg-Pro-Pro-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 5 - Human G34(1-6) ("hG34(6)"): pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 6 - Human G34(13-22) ("hG34/G17 combination"): Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Pro-Pro-Pro-Pro-Cys

Each of these peptides were conjugated to amino groups present on a carrier such as Diphtheria toxoid ("DT") via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent.

To accomplish the linkage between any of Peptides 1-6 above and the carrier, the dry peptide was dissolved in 0.1 M Sodium Phosphate Buffer, pH 8.0, with a thirty molar excess of dithiothreitol ("DTT"). The solution was stirred under a water saturated nitrogen gas atmosphere for four hours. The peptide containing reduced cysteine was separated from the other components by chromatography over a G10 Sephadex column equilibrated with 0.2 M Acetic acid. The peptide was lyophilized and stored under vacuum until used. The carrier was activated by treatment with the heterobifunctional linking agent, e.g., Epsilon-maleimidocaproic acid N-hydroxysuccinimide ester, ("EMCS"), in proportions sufficient to achieve activation of approximately 25 free amino groups per 10^5 molecular weight of carrier. In the specific instance of diphtheria toxoid, this amounted to the addition of 6.18 mg of EMCS (purity 75%) to each 20 mg of diphtheria toxoid.

Activation of diphtheria toxoid was accomplished by dissolving each 20 mg aliquot of diphtheria toxoid in 1 ml of 0.2 M Sodium Phosphate Buffer, pH 6.45. Aliquots of 6.18 mg EMCS were dissolved into 0.2 ml of Dimethyl Formamide ("DMF"). Under darkened conditions, the EMCS was added dropwise in 50 microliter ("ul") amounts to the DT with stirring. After 2 hours of incubation in darkness, the mixture was chromatographed on a G50 Sephadex column equilibrated with 0.1 M Sodium Citrate buffer, pH 6.0, containing 0.1 mM EDTA.

Fractions containing the EMCS activated diphtheria toxoid were concentrated over a PM 10 ultrafiltration membrane under conditions of darkness. The protein content of the concentrate was determined by either the Lowry or Bradford methods. The EMCS content of the carrier was determined by incubation of the activated carrier with cysteine-HCl followed by reaction with 10 mM of Elman's Reagent 5,5'dithio-bis (2-nitrobenzoic acid) 10 mM. The optical density difference between a blank tube containing cysteine-HCl and the sample tube containing cysteine-HCl and carrier was translated into EMCS group content by using the molar extinction coefficient of 13.6×10^3 for 5-thio-2-nitro benzoic acid at 412 nm.

The reduced cysteine content (-SH) of the peptide was also determined utilizing Elman's Reagent. Approximately 1 mg of peptide was dissolved in 1 ml of nitrogen gas saturated water and a 0.1 ml aliquot of this solution was reacted with Elman's Reagent. Utilizing the molar extinction coefficient of 5-thio-2-nitro-benzoic acid (13.6×10^3), the free cysteine -SH was calculated. An amount of peptide containing sufficient free -SH to react with each of the 25 EMCs activated amino groups on the carrier was dissolved in 0.1 M Sodium Citrate Buffer, pH 6.0, containing 0.1 mM EDTA, and added dropwise to the EMCS activated carrier under darkened conditions. After all the peptide solution had been added to the carrier, the mixture was incubated overnight in the dark under a water saturated nitrogen gas atmosphere.

The conjugate of the peptide linked to the carrier via EMCS is separated from other components of the mixture by chromatography over a G50 Sephadex column equilibrated with 0.2 M Ammonium Bicarbonate. The conjugate eluted in the column void volume is lyophilized and stored desiccated at 20°C until used.

The conjugate may be characterized as to peptide content by a number of methods known to those skilled in the art including weight gain, amino acid analysis, etc. Conjugates of these peptides and diphtheria toxoid produced by these methods were determined to have 20-25 moles of peptide per 10^5 MW of carrier and all were considered suitable as immunogens for immunization of test animals.

Example 5

Peptide hG17(1-9)-Ser9 was prepared by standard solid state synthesis methods. That peptide contains an amino terminal immunomimic of hG17 followed by a carboxy terminal spacer. This peptide comprises a 9 amino acid immunomimic of hG17 (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-) followed by the "Ser" spacer (-Ser-Ser-Pro-Pro-Pro-Pro-Cys) attached to amino acid number 9 of the hG17 immunomimic.

The peptide was conjugated to amino groups present on the Diphtheria Toxoid ("DT") immunogenic carder via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent essentially as described in Example 4.

The immunogenic constructs of this invention include an aminoterminal (1-9) G17 peptide or an aminoterminal (1-6) G34 peptide conjugated via a peptide spacer to an immunogenic carrier. The preferred G17 sequence is pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu and the preferred G34 sequence is pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys. The preferred spacer in both constructs is a Ser-peptide (Ser-Ser-Pro-Pro-Pro-Pro-Cys). The preferred immunogenic carrier is diphtheria toxoid, tetanus toxoid, keylimpet hemocyanin, and bovine serum albumin (BSA). The gastrin immunogen is defined as a conjugate of the pGlu- Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu peptide sequence, with an amino acid spacer linked to an immunogenic carrier. The preferred gastrin immunogen is defined as a conjugate of the (1-9) amino terminal (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu) peptide which is linked by peptide spacer to diphtheria toxoid.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

WE CLAIM:

1. A method for the treatment of gastroesophageal reflux disease, comprising administering to a patient an effective amount of an immunogenic composition which generates anti-gastrin antibodies in the patient which bind to gastrin; and administering to said patient an effective amount of histamine H_2 antagonist or a proton pump inhibitor.

2. The method of claim 1, wherein the immunogenic composition is a G17(1-9)Ser9-Diphtheria Toxoid and a pharmaceutically acceptable carrier.

3. The method of claim 1 wherein the histamine H_2 antagonist is administered to the patient until the antibody titer is 10-300pmole/ml.

4. The method of claim 1, wherein the histamine H_2 antagonist is ranitidine.

5. The method of claim 1, wherein the histamine H_2 antagonist is cimetidine.

6. The method of claim 1, wherein the histamine H_2 antagonist is fomatidine.

7. The method of claim 1, wherein the proton pump inhibitor is administered to the patient until the patient's serum anti-G17 antibody titer is 10^{-9}M.

8. The method of claim 1, wherein the proton pump inhibitor is omeprazole.

9. The method of claim 1, wherein the proton pump inhibitor is lansoprazole.

10. The method of claim 1, wherein the proton pump inhibitor is pantoprazole.

11. The method of claim 1 or 7, wherein the anti-gastrin immunity is maintained by a periodic booster of the anti-gastrin immunogenic composition.

12. The method of claim 11, wherein the periodic booster is administered for about a year.

13. Use of an effective amount of an immunogenic composition which generates anti-gastrin antibodies in the patient which bind to gastrin and an effective amount of histamine H_2 antagonist or a proton pump inhibitor in the preparation of a two-component composition for the treatment of gastroesophageal reflux disease, wherein said treatment comprises administering said immunogenic composition and said histamine antagonist or proton pump inhibitor to a patient.

14. Use according to claim 13, wherein the immunogenic composition is a G17(1-9)Ser9-Diphtheria Toxoid and a pharmaceutically acceptable carrier.

15. Use according to claim 13 wherein the histamine H_2 antagonist is administered to the patient until the antibody titer is 10-300 pmole/ml.

16. Use according to claim 13, wherein the histamine H_2 antagonist is ranitidine.

17. Use according to claim 13, wherein the histamine H_2 antagonist is cimetidine.

18. Use according to claim 13, wherein the histamine H_2 antagonist is fomatidine.

19. Use according to claim 13, wherein the proton pump inhibitor is administered to the patient for until the patient's serum anti-G17 antibody titer is 10^{-9}M.

20. Use according to claim 13, wherein the proton pump inhibitor is omeprazole.

21. Use according to claim 13, wherein the proton pump inhibitor is lansoprazole.

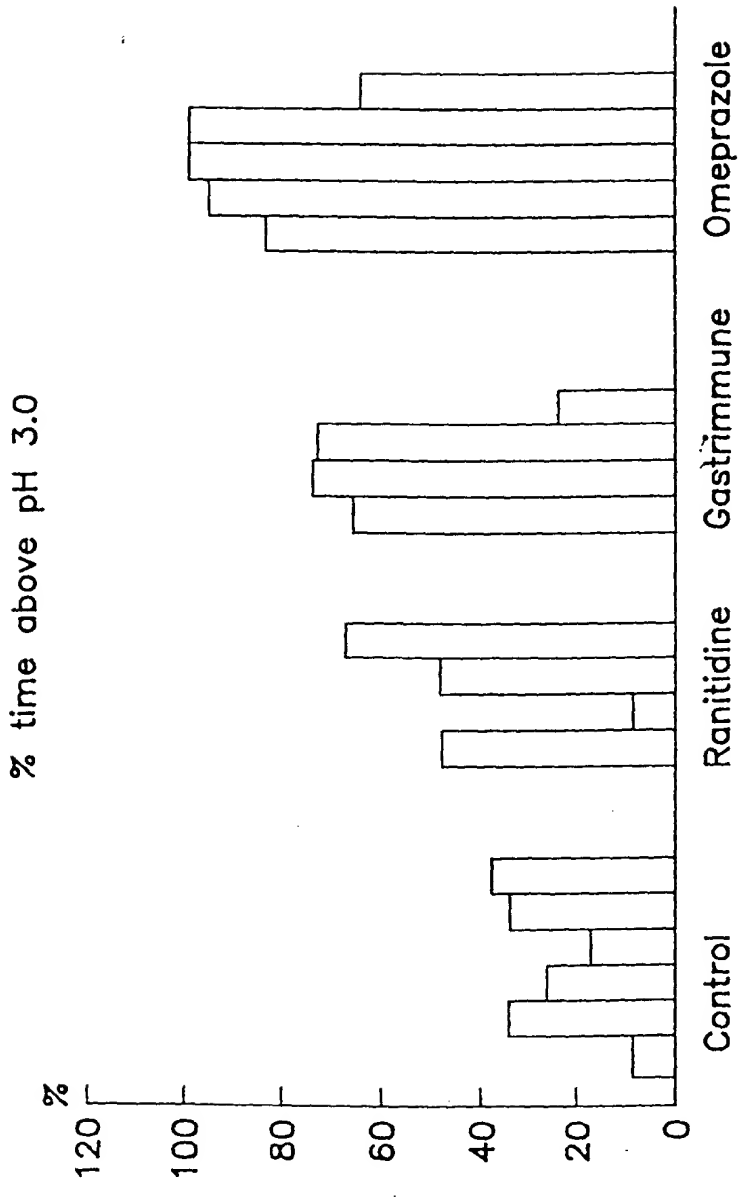
22. Use according to claim 13, wherein the proton pump inhibitor is pantoprazole.

23. Use according to claim 13 or 19, wherein the anti-gastrin immunity is maintained by a periodic booster of the anti-gastrin immunogenic composition.

24. Use according to claim 23, wherein the periodic booster is administered for about a year.

25. A method for the treatment of gastroesophageal reflux disease, comprising administering to a patient an effective amount of purified anti-gastrin antibodies which bind to gastrin; and administering to said patient an effective amount of a histamine H_2 antagonist or a proton pump inhibitor.

26. Use of an effective amount of purified anti-gastrin antibodies which bind to gastrin; and an effective amount of a histamine H_2 antagonist or a proton pump inhibitor in the preparation of a medicament for the treatment of gastroesophageal reflux disease.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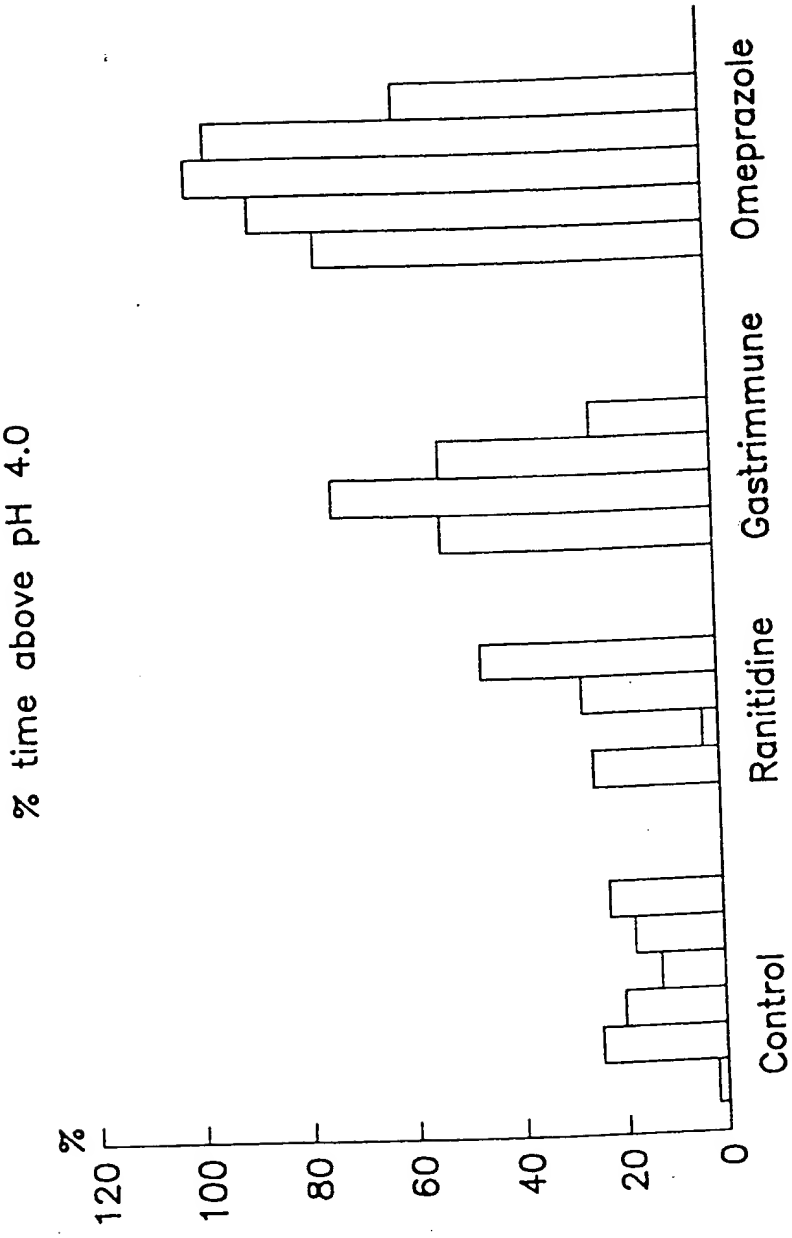
%
120
100
80
60
40
20
0
Control
Ranitidine
Gastrimmune
Omeprazole

FIG. 1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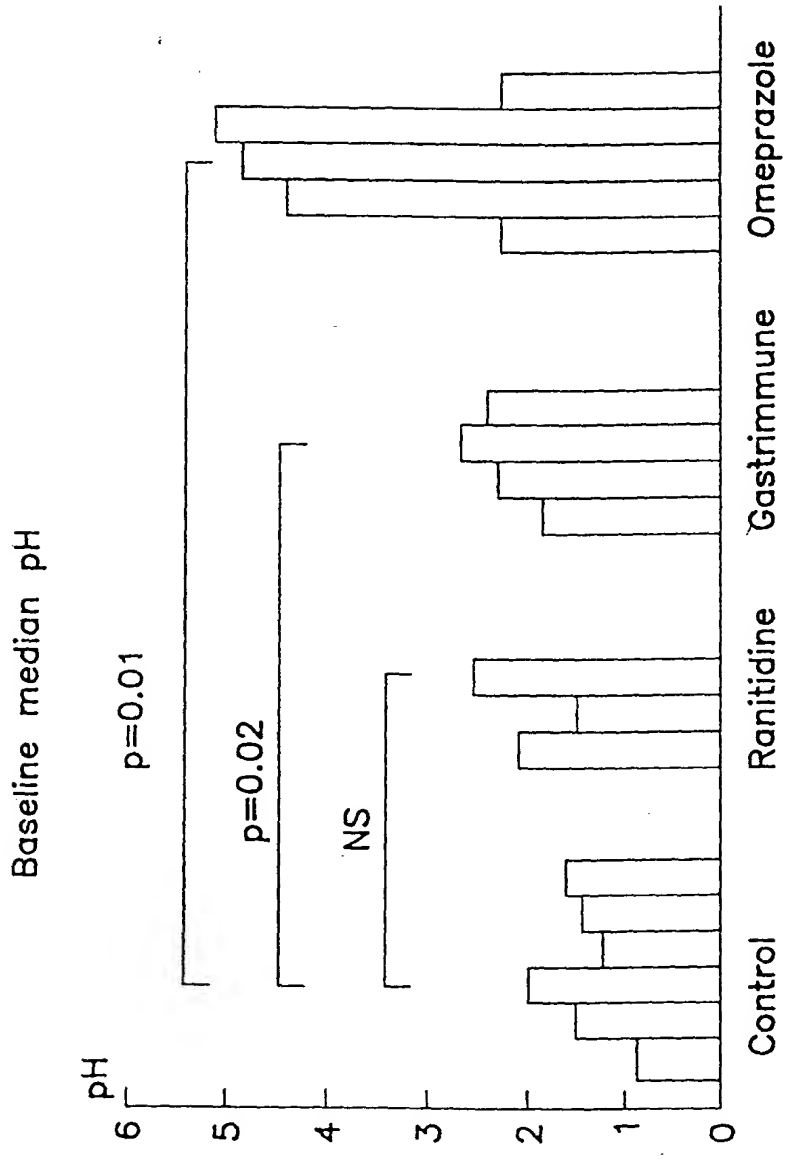
120
100
80
60
40
20
0
Control
Ranitidine
Gastrimmune
Omeprazole

FIG. 2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p=0.02
NS
pH
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5
4
3
2
1
0
Control
Ranitidine
Gastrimmune
Omeprazole

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10734

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 39/00, 39/385, 31/44, 31/415, 31/425

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/13, 14, 15, 16, 17, 18, 338, 339, 370, 400; 424/185.1, 195.11, 197.11, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,468,494 A (GEVAS et al.) 21 November 1995, entire document.	1-26
Y	US 5,609,870 A (GEVAS et al.) 11 March 1997, entire document.	1-26
Y	US 5,023,077 A (GEVAS et al.) 11 June 1991, entire document.	1-26
Y	BUDAVARI, S. The Merck Index (11th Ed.). Rahway, N.J.: Merck & Co., 1989, page 1082.	8-10 and 20-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 JULY 1999	24 AUG 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks	Authorized officer D. Lawrence for

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10734

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/13, 14, 15, 16, 17, 18, 338, 339, 370, 400; 424/185.1, 195.11, 197.11, 198.1